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DESCRIPTION OF DRAWINGS

[Brief Description of the Drawings]

[Drawing 1]The outline of a Japan cedar allergen epitope and connection peptide polymerization protein expression plasmid construction.

[Drawing 2]The mimetic diagram of the polymerization protein revealed by pUC(h6-1)₂, pUC(h7-1)₂, and pUC(h7-3)₂.

[Drawing 3]The outline of pUC18M by RC-PCR method, and pUCM5 construction.

[Drawing 4]The mimetic diagram of the expression control field of expression vector pUC18M and pUCM5.

[Drawing 5]The outline of the pUCM5-h73 construction by RC-PCR method.

[Drawing 6]The outline of the formation of many copies of h7-3 gene including a ribosome coupling region.

[Drawing 7]Comparison of the Homo sapiens T cell growth stimulus activity of cedar pollen allergen origin T cell epitope connection peptide (the peptide 1 and peptide 9) and a T cell epitope mixture.

[Drawing 8]Comparison of the Homo sapiens T cell growth stimulus activity of cedar pollen allergen origin T cell epitope connection peptide (the peptide 2 and peptide 3) and a T cell epitope mixture.

[Drawing 9]Comparison of the Homo sapiens T cell growth stimulus activity of cedar pollen allergen origin T cell epitope connection peptide (the peptide 4 and peptide 10) and a T cell epitope mixture.

[Drawing 10]Comparison of the Homo sapiens T cell growth stimulus activity of cedar pollen allergen origin T cell epitope connection peptide (the peptide 5 and peptide 6) and a T cell epitope mixture.

[Drawing 11]Comparison of the Homo sapiens T cell growth stimulus activity of cedar pollen allergen origin T cell epitope connection peptide (the peptide 11 and peptide 12) and a T cell epitope mixture.

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CLAIMS

[Claim(s)]

[Claim 1]Following-formula (I): $\alpha_1-\alpha_2-\alpha_3-\alpha_4-\alpha_5-\alpha_6-\alpha_7$ (I)

(Among a formula, α_1 differs from α_7 , respectively and) The array number 16 of an array table. Or an amino acid sequence chosen from a group which consists of the array number 7, the array number 17 of an array table, the array number 18 of an array table or the array number 8 and the array number 19 of an array table, the array number 20 of an array table or the array number 21, the array number 22 of an array table, and the array number 23 of an array table. expressing -- peptide including an amino acid sequence expressed, its complex, its derivative, or its polymer.

[Claim 2]In formula (I), α_1 differs from α_7 , respectively, . It is characterized by expressing an amino acid sequence chosen from a group which consists of the array number 7 of an array table, the array number 17 of an array table, the array number 18 of an array table or the array number 8 and the array number 19 of an array table, the array number 20 of an array table, the array number 22 of an array table, and the array number 23 of an array table. The peptide according to claim 1, its complex, its derivative, or its polymer.

[Claim 3]In formula (I), α_1 differs from α_7 , respectively, . It is characterized by expressing an amino acid sequence chosen from a group which consists of the array number 16 of an array table or the array number 7 and the array number 17 of an array table, the array number 8 of an array table, the array number 19 of an array table, the array number 20 of an array table, the array number 22 of an array table, and the array number 23 of an array table. The peptide according to claim 1, its complex, its derivative, or its polymer.

[Claim 4]Peptide which consists of an amino acid sequence shown with the array number 4 of an array table.

[Claim 5]Peptide which consists of an amino acid sequence shown with the array number 5 of an array table.

[Claim 6]Peptide which consists of an amino acid sequence shown with the array number 6 of an array table.

[Claim 7]Peptide which consists of an amino acid sequence shown with the amino acid numbers 1-209 of the array number 12 of an array table.

[Claim 8]Peptide which consists of an amino acid sequence shown with the amino acid numbers 1-96 of the array number 13 of an array table.

[Claim 9]Peptide which consists of an amino acid sequence shown with the amino acid numbers 1-95 of the array number 15 of an array table.

[Claim 10]Following-formula (II): $\alpha_1-\alpha_2-\alpha_3-\alpha_4-\alpha_5-\alpha_6$ (II)

(Among a formula, α_1 differs from α_6 , respectively and) an amino acid sequence chosen from a group which consists of the array number 16 of an array table, the array number 18 of an array table, the array number 19 of an array table, the array number 20 of an array table or the array number 21, the array number 22 of an array table, and the array number 23 of an array table -- expressing -- peptide including an amino acid sequence expressed. The complex, its

derivative, or its polymer.

[Claim 11]Peptide which consists of an amino acid sequence shown with the array number 1 of an array table.

[Claim 12]Peptide which consists of an amino acid sequence shown with the array number 2 of an array table.

[Claim 13]Peptide which consists of an amino acid sequence shown with the array number 3 of an array table.

[Claim 14]Peptide which consists of an amino acid sequence shown with the amino acid numbers 1-185 of the array number 10 of an array table.

[Claim 15]Peptide which consists of an amino acid sequence shown with the array number 7 of an array table.

[Claim 16]Peptide which consists of an amino acid sequence shown with the array number 8 of an array table.

[Claim 17]DNA including a nucleotide sequence which encodes peptide of any one statement of claim 1 thru/or 16.

[Claim 18]Transformation Escherichia coli E.coli pBR(h6-1) SANK 70199 (FERM BP-6642), The E.coli pBR(h7-1) SANK. . It is characterized by being included in a plasmid vector held at any one microorganism chosen from a group which consists of 70299 (FERM BP-6643) and said E.coli pBR(h7-3) SANK 70399 (FERM BP-6644). The DNA according to claim 17.

[Claim 19]DNA including a nucleotide sequence shown in the nucleotide numbers 4-558 of the array number 9 of an array table.

[Claim 20]DNA including a nucleotide sequence shown in the nucleotide numbers 4-630 of the array number 11 of an array table.

[Claim 21]DNA including a nucleotide sequence shown in the nucleotide numbers 4-288 of the array number 14 of an array table.

[Claim 22]A recombinant vector containing DNA of any one statement of claim 17 thru/or 21.

[Claim 23]The recombinant vector according to claim 22, wherein DNA of any one statement of claim 17 thru/or 21 is incorporated into a vector which closes a manifestation of peptide which consists of an amino acid sequence by which a code is carried out to this DNA if possible.

[Claim 24]A host cell holding the recombinant vector according to claim 22 or 23.

[Claim 25]Transformation Escherichia coli E.coli pBR(h6-1) SANK 70199 (FERM BP-6642), . It is characterized by being any one microorganism chosen from a group which consists of said E.coli pBR(h7-1) SANK 70299 (FERM BP-6643) and said E.coli pBR(h7-3) SANK 70399 (FERM BP-6644). The host cell according to claim 24.

[Claim 26]A host cell holding the recombinant vector according to claim 23 is cultured under conditions which can produce peptide which a recombinant DNA included in this vector encodes, Subsequently, a manufacturing method of peptide of any one statement of claim 1 thru/or 16 collecting this peptide from this culture.

[Claim 27]A host cell transformed by a recombinant vector including a nucleotide sequence which is a manufacturing method of the peptide according to claim 8, and is shown in the array number 60 of one array table, a process cultivated under conditions which can reveal peptide which consists of an amino acid sequence by which a code is carried out to a nucleotide sequence shown by the nucleotide numbers 1-642 in this nucleotide sequence — it ranking second two and, a process of collecting peptide from a culture obtained by 1), and 3 — a method of including a process of collecting the peptide according to claim 8, after carrying out trypsin digestion of the peptide obtained by 2 further.

[Claim 28]An anti-hay fever agent which contains peptide of any one statement of claim 1 thru/or 16, its complex, its derivative, or its polymer as an active principle.

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DETAILED DESCRIPTION

[Detailed Description of the Invention]

[0001]

[Field of the Invention]This invention relates to the peptide which inactivates the Homo sapiens T cell which reacts to cedar pollen allergen specifically, and the immunotherapy agent which contains the peptide as an active principle.

[0002]

[Description of the Prior Art]If at the beginning of spring has come in our country since here tens years, the number of those who appeal against the rhinitis and the conjunctivitis by hay fever will continue increasing. Hay fever is a kind of an allergy and it is said that the main factor is, the antigenic substance, i.e., the cedar pollen allergen, in cedar pollen. If the cedar pollen which dispersed in the atmosphere trespasses upon the human inside of the body, the immunoglobulin E antibody to cedar pollen allergen will be produced. When cedar pollen invades next in this state, the allergen and this immunoglobulin E antibody in that pollen will start immunoreaction, and will present allergies.

[0003]It is known by the present that at least two kinds of antigenic different allergen exist in cedar pollen. One of them is protein called "Cryj1" reported to the literature [J. Allergy Clin. Immunol. 71 and 77-86] (1983) of YASUEDA and others.

Another is protein called "Cryj2" reported to the literature [FEBS Letters, 239, and 329-332] (1988) of TANIAI and others, and the literature [allergy, No. 45, and 309 - 312 pages (1990)] of Sakaguchi and others.

The whole-amino-acid arrangement is determined for Cryj1 and Cryj2 by the present [refer to the No. WO93/01213, and Patent Publication Heisei 8-505284 gazette].

[0004]Into cedar pollen, Cryj1 and Cryj2 exist at a rate of about 50:1 thru/or 5:1, and they are usually said for most blood serums extracted from the hay fever sufferer to react also to Cryj1 and Cryj2. In the intracutaneous-reaction examination or the RAST examination, it is reported to the literature [allergy, 42nd volume, and 738 - 747 pages (1993)] of Sawatani and others that Cryj1 and Cryj2 demonstrate comparable antigenicity.

[0005]Thus, since cedar pollen allergen already isolated partly and the character and description were also solved to some extent, the prospect which can treat and prevent hay fever followed by medicating Homo sapiens with refining cedar pollen allergen, and carrying out hyposensitization. These days, some hyposensitization agents for it are also devised, for example, JP,1-156926,A and JP,3-93730,A carry out the covalent bond of the sugar to cedar pollen allergen, and the proposal with which Homo sapiens is medicated by making the generated complex into a hyposensitization agent is made.

[0006]However, the allergen of a high grade is usually needed for a large quantity, and the allergen in cedar pollen has low stability to a small top, and if it tries to provide the diagnostic agent and hyposensitization agent of hay fever only with cedar pollen, great difficulty will follow it on diagnosis and the desensitization therapy of an allergy. In the therapy and prevention of the allergosis latest since it is such, Like the former, a patient is not medicated with the whole allergen but the minimal domain which the T cell in allergen recognizes specifically, i.e., the immunotherapy which prescribes for the patient the low-molecular peptide which consists only

of T cell epitopes intrinsically, attracts attention.

[0007] Generally, when allergen is incorporated into antigen presenting cells, such as a macrophage, it is digested there, and a digestive fragment will join together and antigen presentation will be carried out to HLA (Human Leukocyte Antigen) protein of an antigen presenting cell surface. The field which the fragment by which antigen presentation is carried out is restricted to some [in allergen] specific regions by the compatibility over HLA protein, etc., and a T cell recognizes specifically among these fields is usually called a "T cell epitope." In the immunotherapy which prescribes for the patient the peptide which consists only of T cell epitopes substantially, (i) Peptide lacks the B cell epitope, namely, since a specific immunoglobulin E antibody does not react to allergen, side effects, such as anaphylaxis which had occurred frequently with the conventional poor quality or refining allergen, cannot happen.; (ii) A period until it starts from a small quantity and reaches an effective dose can be substantially shortened as compared with the conventional hyposensitization agent.; (iii) Oral tolerance is derived and there is an advantage of being able to decrease the allergic reaction to allergen. However, since it may be ineffective even if all hay fever patients do not react to a common T cell epitope uniformly and prescribe only one kind of T cell epitope for the patient, A positive effect is not expectable, if a medicine is prescribed for the patient after a patient investigates first to which T cell epitope it reacts, or two or more T cell epitopes are prepared and they are not simultaneously prescribed for the patient. However, by the former method, it must investigate to which T cell epitope a patient reacts beforehand, and two or more T cell epitopes must be independently prepared by the latter method.

[0008] Then, the trial which uses the peptide which connected several different T cell epitopes artificially is made in recent years [for example, refer to the No. Patent Publication Heisei 7-503362 gazette]. Such a connection T cell epitope is produced also cedar pollen allergen [refer to JP,10-259198,A].

[0009]

[Problem(s) to be Solved by the Invention]. The first technical problem of this invention excelled what is known conventionally in bringing a wide range person the prevention or the curative effect over hay fever by a low dose. It is in providing the peptide which consists of an amino acid sequence which the amino acid sequence of several Homo sapiens T cell epitopes from which the cedar pollen allergen origin differs followed.

[0010] There is the second technical problem of this invention in providing DNA which encodes the above-mentioned peptide. There is the third technical problem of this invention in providing the manufacturing method of the above-mentioned peptide. There is the fourth technical problem of this invention in providing the anti-hay fever agent which contains the above-mentioned peptide as an active principle.

[0011]

[Means for Solving the Problem] This invention persons newly limited the range of a T cell epitope excellent in especially an effect as a desensitization therapy agent to hay fever. Peptide which can demonstrate prevention or a curative effect of hay fever which was superior to what was known conventionally to a wide range person was produced using these T cell epitopes. On the other hand, peptide of this invention into which amino acid with this invention persons unrelated among the connected T cell each epitopes does not go showed clearly that a therapy or a preventive effect is expressed with a low dose to immunotherapy of hay fever rather than what only mixed each T cell epitope. And this invention persons provided prevention or a treating agent of hay fever which makes these peptide an active principle, and completed this invention.

[0012] That is, this invention is following formula (I): α_1 - α_2 - α_3 - α_4 - α_5 - α_6 - α_7 (I).

(Among a formula, α_1 differs from α_7 , respectively and) The array number 16 of an array table. Or an amino acid sequence chosen from a group which consists of the array number 7, the array number 17 of an array table, the array number 18 of an array table or the array number 8 and the array number 19 of an array table, the array number 20 of an array table or the array number 21, the array number 22 of an array table, and the array number 23 of an array table.

expressing -- peptide including an amino acid sequence expressed, its complex, its derivative, or polymer;

Following-formula (II): $\alpha_1\text{-}\alpha_2\text{-}\alpha_3\text{-}\alpha_4\text{-}\alpha_5\text{-}\alpha_6$ (II)

(Among a formula, α_1 differs from α_6 , respectively and) an amino acid sequence chosen from a group which consists of the array number 16 of an array table, the array number 18 of an array table, the array number 19 of an array table, the array number 20 of an array table or the array number 21, the array number 22 of an array table, and the array number 23 of an array table -- expressing -- peptide including an amino acid sequence expressed. The complex, its derivative, or its polymer; it is related, without peptide which consists of an amino acid sequence shown with the array number 8 of peptide; and an array table which consists of an amino acid sequence shown with the array number 7 of an array table.

[0013]Hereafter, this invention is explained in detail. 6 thru/or 7 kinds chosen one [at a time] from A group which defines this invention below, B, C group, D, E group, and each amino acid sequence group of F and G, What connected seven kinds of amino acid sequences in random order more preferably, its complex, :A group which is what provides the derivative or its polymer: Amino acid sequence shown in the array number 7 of an amino acid sequence (henceforth "A") shown in the array number 16 of an array table, or an array table (henceforth "A'");

B: Amino acid sequence shown in the array number 17 of an array table;

C group: Amino acid sequence shown in the array number 8 of an amino acid sequence (henceforth "C") shown in the array number 18 of an array table, or an array table (henceforth "C'");

D: Amino acid sequence shown in the array number 19 of an array table;

E group: Amino acid sequence shown in the array number 21 of an amino acid sequence (henceforth "E") shown in the array number 20 of an array table, or an array table (henceforth "E'");

F: Amino acid sequence shown in the array number 22 of an array table;

G: An amino acid sequence shown in the array number 23 of an array table.

[0014]moreover -- this invention -- being new -- Homo sapiens -- a T cell epitope -- constituting -- an amino acid sequence -- having -- hay fever -- receiving -- a desensitization therapy -- an agent -- an active principle -- ***** -- being useful -- the above -- an amino acid sequence -- A -- ' -- or -- C -- ' -- having -- peptide -- providing . Here with "an amino acid sequence which constitutes the Homo sapiens T cell epitope." When a peripheral blood mononuclear cell population of hay fever patient origin is cultivated under existence of peptide which has this amino acid sequence, An amino acid sequence which has speed exceeding the twice of a peripheral blood mononuclear cell population which cultivated DNA synthesis speed of this peripheral blood mononuclear cell population under nonexistence of peptide which has this amino acid sequence, and the activity more preferably made into speed of 5 times or more is said. In the above, E group, and F and G are the groups of an amino acid sequence which constitutes Homo sapiens main T cell epitopes of the cedar pollen allergen Cryj1 origin, and others are all the groups of an amino acid sequence which constitutes Homo sapiens main T cell epitopes of the Cryj2 origin.

[0015];peptide 1 (array number 1 of an array table) which can give the following example as desirable peptide in this invention;

Peptide 2 (array number 2 of an array table);

Peptide 3 (array number 3 of an array table);

Peptide 4 (array number 4 of an array table);

Peptide 5 (array number 5 of an array table);

Peptide 6 (array number 6 of an array table);

Peptide 7 (array number 7 of an array table);

Peptide 8 (array number 8 of an array table);

Peptide 9 (amino acid numbers 1-185 of the array number 10 of an array table);

Peptide 10 (amino acid numbers 1-209 of the array number 12 of an array table);

Peptide 11(array number 13 of array table);, and peptide 12 (amino acid numbers 1-95 of the

array number 15 of an array table).

[0016]The above-mentioned peptide 1 has the amino acid sequence with which an amino acid sequence which constitutes Homo sapiens main T cell epitopes of cedar pollen allergen protein was connected in order of A-E-C-D-G-F from the end of N. ;peptide 2 in which similarly the peptide 2 thru/or 6 has the following structures: A-E'-C-D-F-G;

Peptide 3: A-E'-C-D-G-F;

Peptide 4: B-A-E-C-D-F-G;

Peptide 5: B-A'-E-C'-D-F-G.

[0017]The peptide 6 has the amino acid sequence to which arrangement Gly-Asp-Pro-Arg which is from amino acid 4 residue on an amino terminal side of the peptide 5 was added. The peptide 7 and the peptide 8 are above-mentioned A' and peptide itself which have an amino acid sequence of C', respectively.

[0018]A unit which has the structure where the peptide 9 is expressed with A-E-C-D-F-G (henceforth "H6-1") The amino acid numbers 4-84 of the array number 25 of an array table, The following amino acid sequence. Linker peptide which it has : Thr-Met-Ile-Thr-Asn-Ser-Ser-Ser-Val-Pro-Gly-Asp-Pro-Arg(L1, array number 26 of array table);Arg-Ala-Asp-Pro-Arg. (L2, array number 27 of an array table); and Arg-Ala-Asp-Leu (L1), -(H6-1)-(L2)-(H6-1)- (L3) (L3, the array number 80 of an array table)

** -- it has the polymeric structure connected like. These linkers of cedar pollen allergen are unrelated, in order to make Escherichia coli produce a polymer of H6-1 simple using recombinant DNA technology, it is added or inserted, but the activity of the peptide 9 itself is not affected. A unit which similarly has the structure where the peptide 10 is expressed with A-E-C-D-F-G-B (henceforth "H7-1") The array number 29 and the above-mentioned linker peptide of an array table (L1), -(H7-1)-(L2)-(H7-1)- (L3)

** -- it has the structure connected like.

[0019]Although a short linker intervenes, peptide which has these polymeric structure has the effect same as mentioned above as a monomer, and has the advantage that production is possible, that it is simple and in large quantities by recombinant DNA technology. Although it is usable as it is, it can decompose and this polymer can also be used as a monomer, so that a next example may indicate. The peptide 12 has the structure expressed with A'-E-C'-D-F-G-B, and the peptide 11 has the structure where Arg was added to a C terminal of the peptide 12.

[0020]Permutation, for example, A'-B-C'-D-E-F-G, G-F-E-D-C' of T cell epitopes other than an above mentioned thing - B-A' etc. are preferred as peptide of this invention. Although permutation of seven sorts of T cell epitopes of B, C', D, E, F, and G will be $7 \times 6 \times 5 \times 4 \times 3 \times 2 \times 1 = 5040$ kind including the above and A' and the peptide which connected these T cell epitopes can take 5040 kinds of structures, Since T cell epitope activity should just be positive and the permutation in particular itself is not limited when peptide of this invention is investigated by a method of measuring incorporation of tritition thymidine of a T cell specific to cedar pollen allergen mentioned later, those peptide is also included by this invention.

[0021]It is peptide chosen from a group which a more suitable thing becomes from the peptide 4, 5, 6, 10, 11, and 12 in this invention among the above-mentioned peptide, Especially a suitable thing is peptide chosen from a group which consists of the peptide 5, 6, 11, and 12, and the most suitable thing is the peptide 11 or 12.

[0022]Peptide of this invention in the above composition to in addition, an amino terminal and/or a C terminal of what connected a T cell epitope. That in which an additional amino acid sequence unrelated to composition of a T cell epitope exists is also included (for example, Arg etc. of arrangement expressed with Gly-Asp-Pro-Arg of an amino terminal of the peptide 6, a linker of the peptide 9 or 10, and a C terminal of the peptide 11). Structure of such an additional amino acid sequence is not limited as long as it seems that it does not have some harmful activity, such as toxicity, when a thing which spoils a function as a T cell epitope donor of peptide of this invention, or Homo sapiens is medicated.

[0023]Especially the peptide 11 is obtained by a manufacturing method concerning this invention indicated later, and even if Arg adds, it has the same effect as the peptide 12. This invention persons examined various methods of obtaining peptide of this invention, and in order to attain

the purpose of this invention, they found out suitable DNA, a recombinant vector, a host cell, and a manufacturing method of peptide. This invention persons had an effective anti-hay fever agent which contains peptide concerning this invention as an active principle to a more extensive hay fever patient, and have activity by a low dose and found out that a dose could be lessened.

[0024]

[Embodiment of the Invention]In the field known as a "solid phase technique" or a "liquid phase process", peptide given in this invention can be prepared by a conventional peptide synthesis method. For example, the details of peptide synthesis are indicated for the Tokyo Kagaku Dojin issue in the edited by Japanese Biochemical Society "new chemical experiment lecture", the 1st volume, "protein VI", the 3-44th page, and 1992. A multi-peptide synthesizer (a symphony, protein technology company make) is used for this peptide, and it is Fmoc. According to the protocol of the device, it is compoundable by a solid-phase-synthesis (9-fluorenyl methyloxycarbonyl) method. That is, the Fmoc-L-amino acid Wang resin (or Cl-Trt resin) in which the amino acid equivalent to the C terminal of each peptide to compound is introduced is set to the reaction vessel of the above-mentioned peptide synthesis device, and Fmoc is excluded using a deprotection solution. Target peptide is compoundable by making the amino acid solution and activator solution which are furthermore equivalent to the 2nd amino acid from a C terminal react, performing deprotection of a Fmoc basis again after a reaction, and repeating the same operation. In the above-mentioned method, when the peptide which it is generally going to compound becomes long, there is a tendency for yield to fall, but. As shown in the following example, it is possible to maintain high yield by inactivating some C-terminal amino acid beforehand introduced into resin, and reducing the number of the C-terminal amino acid in which future reactions are possible.

[0025]Peptide of this invention is not limited to what was prepared by chemosynthesis, but may be prepared by recombinant DNA technology, For example, DNA which encodes either of the above-mentioned peptide 1 thru/or 12 is prepared, and what inserted this in the vector in which independence growth is possible may be introduced into hosts, such as Escherichia coli, bacillus subtilis, a ray fungus, and yeast, it may be considered as a transformant, and peptide of this invention may be extracted from the culture.

[0026]Therefore, this invention provides DNA including the nucleotide sequence which encodes peptide of above-mentioned this invention. In this invention, what is included in the plasmid vector held especially at the below-mentioned transformation Escherichia coli can use it conveniently. As another viewpoint, this invention provides the recombinant vector containing the above-mentioned DNA. In this invention, what is incorporated into the vector which closes the manifestation of the peptide which consists of an amino acid sequence by which a code is carried out to the above-mentioned DNA as a recombinant vector if possible can use it conveniently.

[0027]As a method of preparing DNA which has a desired nucleotide sequence, For example, chemosynthesis of a sense and an antisense nucleotide which it is a partial sequence nucleotide of DNA of this request, and both ends overlap is carried out, Subsequently, the method of obtaining what these partial sequences connected, etc. are mentioned by using DNA polymerase reactions and ligase reactions, such as polymerase chain reaction [Saiki, R. K. et al (1988) Science 239, and 487 -491 reference].For example, as a microorganism transformed by the vector in which DNA which encodes peptide of this invention by which artificial composition was carried out by making it such was included, The vector in which DNA which encodes the peptide 9 was included, the vector in which DNA which encodes the peptide 10 was included, It reaches, The peptide 11 or the peptide 12. Escherichia coli stock E.coli pBR(h6-1) SANK 70199, E.coli pBR(h7-1) SANK 70299, and E.coli pBR (h7-3) which were transformed by the vector in which DNA to encode was included, respectively. The international deposit of SANK 70399 is carried out to National Institute of Bioscience and Human-Technology on February 9, 1999, and accession number FERM BP-6642, FERM BP-6643, and FERM BP-6644 are attached, respectively. Although the most suitable thing as a DNA which encodes peptide of this invention can be obtained from the above-mentioned deposition strain with the usual gene engineering technique, DNA which encodes peptide of this invention is not limited to these.

[0028]The host cell of a procaryote or eukaryote can be made to transform like the above by including DNA which encodes the amino acid sequence of peptide of this invention produced by carrying out in a suitable vector. This DNA can be made to reveal in each host cell by introducing the arrangement in connection with the suitable promotor and transformation for these vectors. That is, this invention relates to the host cell holding the recombinant vector by which DNA of this invention is incorporated into the vector which closes the manifestation of peptide of this invention again if possible.

[0029]As a host of a prokaryotic cell, *Escherichia coli* (*Escherichia coli*), *Bacillus subtilis* (*Bacillus subtilis*), etc. are mentioned, for example. What is necessary is just to make a host cell transform by the plasmid vector include the replicon, i.e., the replication origin, and the regulatory sequence of the seed origin which may suit with a host, in order to carry out expression of the target gene within these host cells. As for a vector, what has the arrangement which can give the selectivity of the quality of phenotype (phenotype) in a transformed cell is desirable. For example, although 12 shares of *E. coli* K, 109 shares of JM, etc. are well used as *Escherichia coli* and the plasmid of a pBR322 and pUC system is generally well used as a vector, it is not limited to these but each of various kinds of publicly known strains and vectors can use.

[0030]As a promotor, in *Escherichia coli*, a tryptophan (*trp*) promotor, A lactose (*lac*) promotor, a tryptophan lactose (*tac*) promotor, A lipoprotein (*lpp*) promotor, the lambda (*lambda*) PL promotor of bacteriophage origin, A polypeptide chain elongation factor Tu (*tufB*) promotor, *lacUV5* promotor, etc. are mentioned, and any promotor can use it for production of peptide of this invention.

[0031]Although 207 to 25 shares are preferred and pTUB228 [Ohmura, K., et al. (1984) J. Biochem. 95, and 87–93 reference] etc. is used as a vector as *Bacillus subtilis*, for example, it is not limited to this. When the regulatory sequence of the alpha-amylase gene of *Bacillus subtilis* is used well and connects the DNA sequence which encodes the transit peptide arrangement of alpha-amylase as occasion demands further as a promotor for *Bacillus subtilis*, a secretion manifestation out of a biomass is also attained.

[0032]If the case where *Escherichia coli* is used as a host cell is mentioned as an example, as an expression vector, it has pBR322 replication origin, and in *Escherichia coli*, autonomous replication is possible, and what was further provided with the transcriptional promoter and the initiation signal can be used. This expression vector The calcium chloride method [Mandel, M. and Higa, A. (1970) J. Mol. Biol. 53, and 154 references], Method [Hanahan of Hanahan, D. and Meselson, M. (1980) Gene 10, and 63 by reference], the electric pulse terebration [Neumann, E., et al. EMBO J(1982). 1, and 841–845 reference], etc. It can be made to be able to incorporate into *Escherichia coli* and a desired vector can obtain the transfected cell in this way.

[0033]They are contained in the host cell of eukaryote by cells, such as a vertebrate, an insect, and yeast, and as a vertebrate cell, For example, COS cell [Gluzman which is a cell of an ape, (1981) Y. Cell23 and 175–182. The dihydrofolate reductase deficit stock of reference] or a Chinese hamster ovary cell (CHO) [Urlaub, G. and Chasin, and L. A. (1980) Proc. Natl. Acad. Sci. USA 77 and 4216 –4220 reference], Although the *Homo sapiens* Namalwa cell, a hamster BHK cell, etc. are used well, it is not limited to these.

[0034]As an expression vector of a vertebrate cell, what has a promotor located upstream of the gene which you are going to make it usually reveal, a splice site of RNA, a polyadenylation site, conclusion arrangement of transfer, etc. can be used, and this may have a replication origin as occasion demands further. Although pSV2dhfr [Subramani, S., et al. (1981) Mol. Cell. Biol. 1, and 854 –864 reference] etc. which have an initial promotor of SV40 can be illustrated as an example of this expression vector, it is not limited to this.

[0035]Generally as a eucaryotic microorganism, yeast is used well, and a yeast genus *Saccharomyces* (*Saccharomyces cerevisiae*), for example, *Saccharomyces cerevisiae*, is preferred also in it. As an expression vector of eukaryotes, such as this yeast, For example, promotor [Bennetzen of an alcoholic dehydrogenase gene, J. L. and Hall and B. D. (1982) J. Biol. Chem. 257 and 3018–3025 Reference], the promotor [Miyanochara, A., et al.(1983) Proc. Natl. Acad. Sci. USA 80, and 1–5 reference] of an acid phosphatase gene, etc. can be used preferably.

[0036]As a host cell, if the case where a COS cell is used is mentioned as an example, as an expression vector, it has an SV40 replication origin, and in a COS cell, independence growth is possible, and what was further provided with the transcriptional promoter, the transfer concentration signal, and the RNA splice site can be used. This expression vector The DEAE-dextran process [Luthman, H. and Magnusson, G. (1983) *Nucleic Acids Res.* 11, and 1295-1308 reference], Calcium phosphate DNA coprecipitation method [Graham, F. L. and van der Ed, A. J. (1973) *Virology* 52 and 456-457 It can be made to incorporate into a COS cell by reference], the electric pulse terebration [Neumann, E., et al. (1982) *EMBO J.* 1, and 841-845 reference], etc., A desired transformed cell can be obtained in this way. In using a CHO cell as a host cell, The vector which may reveal the neo gene which functions as a G418 tolerance marker with an expression vector, For example, pRSVneo [refer to the Sambrook, J., et al. (1989) "Molecular Cloning: A Laboratory Manual" Cold Spring Harbor Laboratory, NY] and pSV2. neo [Southern, P. J. and Berg, P. (1982) *J. Mol. Appl. Genet.* 1, and 327-341 reference] etc. are KO and transfected, The transformed cell which produces peptide of this invention stably can be obtained by choosing the colony of G418 tolerance.

[0037]As mentioned above, if a host cell is transformed in this invention so that peptide of this invention may be produced, any may be sufficient and are not restricted in particular, but as indicated previously, for example, The vector in which DNA which encodes the peptide 9 was included, the vector in which DNA which encodes the peptide 10 was included, It reaches, The peptide 11 or the peptide 12. *Escherichia coli* stock *E.coli* pBR (h6-1) SANK70199, *E.coli* pBR (h7-1) SANK 70299, and *E.coli* pBR (h7-3) which were transformed by the vector in which DNA to encode was included, respectively. The international deposit of SANK 70399 (accession number FERMBP-6642, FERM BP-6643, and FERM BP-6644) has already been carried out, and it can be conveniently used in operation of this invention.

[0038]The transformant of the request obtained above can be cultivated in accordance with a conventional method, and the polypeptide of this invention is produced out of intracellular or a cell by this culture. Various kinds of things commonly used as a culture medium used for this culture according to the adopted host cell can be chosen suitably, For example, if it is *Escherichia coli*, a trypton yeast culture medium (bacto trypton 1.6% and yeast extract 1.0%, sodium chloride 0.5% (pH 7.0)), a peptone medium (made by Difco), etc. can be used. If it is the above-mentioned COS cell, what added serum components, such as fetal calf serum (FBS), if needed to culture media, such as RPMI1640 culture medium and the Dulbecco correction Eagle's medium (DMEM), can be used.

[0039]Peptide of this invention produced out of intracellular [of a transformant] or a cell by the above can be separated and refined by various kinds of publicly known separating operation methods for having used a physical property, chemical nature, etc. of this protein. Processing according to the concrete for example, usual protein precipitation reagent as this method, Various chromatography, such as ultrafiltration, a molecular sieve chromatography (gel filtration), adsorption chromatography, ion exchange chromatography, affinity chromatography, and high performance chromatography (HPLC), dialysis, these combination, etc. can be illustrated. When a foreign gene is introduced into *Escherichia coli* etc. and an extensive manifestation is carried out, the produced peptide may form a conglomerate insoluble to the water called an inclusion body. In such a case, this peptide can be solubilized by denaturing this peptide using powerful denaturing agents, such as a guanidineisothiocyanate.

[0040]the gestalt as a complex produced by peptide of this invention adding sugar and a polyethylene glycol to the peptide obtained in this way — further, It may be a gestalt as a derivative or a polymer acquired by making carry out crosslinking polymerization of the peptide with acetylation, amidation, and/or a polyfunctional reagent. In manufacture of the complex of such peptide of this invention, a derivative, or a polymer, Addition of sugar etc., acetylation, amidation, and/or crosslinking polymerization, It is preferred to be carried out in additional peptide of the amino terminal of peptide of above mentioned this invention and/or a C terminal or linker peptide so that the function of the amino acid sequence which constitutes the Homo sapiens T cell epitope of the cedar pollen allergen protein origin in peptide of this invention may not be spoiled.

[0041]As a complex of peptide of this invention, for example to the amino group of an amino terminal or Lys residue, to a polyethylene glycol, a monomethoxy polyethylene glycol, dextran, and a pan. What added the polysaccharide which makes maltotrioses, such as pullulan and ERUSHINAN, a repeating unit, A well-known thing can be mentioned to the person skilled in the art in the technical field of this invention, and these can be manufactured according to the statement of the 1st volume of Japanese Biochemical Society editing [the 236-252nd pages (1991, Tokyo Kagaku Dojin issue) of] "new chemical experiment lecture" "protein IV" etc.

[0042]As a derivative of peptide of this invention, what acetylated the amino terminal, for example, the thing which amidated C terminal Gly residue, etc. can mention a well-known thing to the person skilled in the art in the technical field of this invention. this derivative -- the 1st volume of the 18-20th pages of the above "new chemical experiment lecture" "protein IV" -- and -- said -- the 9th volume "hormone I" -- it can manufacture according to the statement of the 290-298th page (Tokyo Kagaku Dojin will publish all in 1991) etc. As a polymer of peptide of this invention, what polymerized the peptide dyad of this invention, for example with bivalency crosslinking reagents, such as a JISUKUSHINIMIJIRUSUBE rate, can mention a well-known thing to the person skilled in the art in the technical field of this invention. Preparation of this polymer can be performed, for example according to the statement of the 1st volume of the above [the 207-226th pages of] "new chemical experiment lecture" "protein IV."

[0043]In this invention, the manufacturing method of peptide by DNA recombination art is provided. The method of obtaining a monomer simple is provided from DNA which encodes the peptide which has especially polymeric structure. For example, after making hosts, such as Escherichia coli, produce a polymer which peptide of this invention has connected via the linker peptide which includes specifically the amino acid sequence which can be cut by protease, peptide of this invention can also be obtained by digesting this polymer by this protease. Although any of the hetero polymer in which the gay polymer in which one of the peptide of this invention polymerized, or peptide of two or more sorts of this inventions polymerized may be sufficient as this polymer in that case, a gay polymer is chosen when aiming at making single peptide produce in large quantities. If a specific amino acid sequence is recognized and cut, each publicly known thing can be used for protease, but it is trypsin, cathepsin B, cathepsin D, the cathepsin E, etc. suitably.

[0044]Even if it prescribes a medicine for the patient with a comparatively rough gestalt, expected therapy and preventive effect are demonstrated, but peptide of this invention is usually refined in advance of use. In refining, for example Filtration, concentration, centrifugal separation, gel filtration chromatography, What is necessary is to use the method of the common use in the field for refining peptide thru/or protein, such as ion exchange chromatography, high performance chromatography, affinity chromatography, gel electrophoresis, and isoelectric focusing, and just to combine these methods suitably if needed. And liquefied or what is necessary is to condense refined peptide, to freeze-dry according to an end-use gestalt, and just to use a solid state. It can check that peptide of this invention has the activity as a T cell epitope by measuring the incorporation of tritition thymidine of a specific T cell to cedar pollen allergen. The method of indicating below, for example can be used for this measurement.

[0045]That is, a hay fever patient's peripheral blood mononuclear cell population is separated by a ficoll High Pack specific gravity centrifuge method etc., the culture medium of RPMI1640 grade is made to float and this cell population is poured distributively on 96 hole microplate. Next, peptide which is a specimen material is added and cultivated. Although conditions, such as temperature at the time of this culture and time, can be suitably adjusted for every experiment, 37 ** and three days are preferred. The activity as a T cell epitope of peptide of this invention is reckonable by adding tritition thymidine to a culture medium after that, continuing fixed time culture further, and measuring the amount of incorporation of tritition thymidine in a mononuclear cell group. In this invention, the system which does not contain peptide simultaneously was provided, this was made into the negative control, the system to which the amount of incorporation of tritition thymidine reached the value exceeding the twice of a negative control was made into the "positivity", and the system which was not attained was made into "negativity."

[0046]It can check that peptide of this invention has a curative effect over hay fever, for example by the following experiments. peptide of this invention — the Homo sapiens T cell — the effect not showing up, even if it experiments for animals other than Homo sapiens since it has specific activity, but. For example, since the lymphocyte of a C3 H/HeN mouse has reactivity to the peptide included by the above-mentioned A group, the method that the amino acid sequence of A group is indicated below about peptide of certainly included this invention can estimate the effect.

[0047]Peptide of this invention is beforehand prescribed for the patient to a C3 H/HeN mouse, and the immunological tolerance to this peptide is derived. The amino acid sequence indicated to the mouse concerned after fixed time progress below. Homo sapiens main T cell epitope peptide of Cryj2 which it has : Gly-Ile-Ile-Ala-Ala-Tyr-Gln-Asn-Pro-Ala-Ser-Trp (peptide 13; array number 16 of an array table) with adjuvants, such as Freund's complete adjuvant. Immunity is prescribed a medicine for the patient and carried out. After fixed time progress, a lymph node cell is extracted from the hollow of knee of the mouse concerned, etc., and cell suspension is prepared. Growth of a T cell can be measured by adding the above-mentioned peptide 13 or Cryj2 to this, continuing culture, adding tritition thymidine further, and measuring the amount of incorporation of tritition thymidine.

[0048]Beforehand, with peptide of this invention, with the mouse which is not deriving immunological tolerance, a specific T cell is activated by the peptide by immunization by the peptide 13, and it reacts to the peptide 13 shown by the antigen presenting cell in in vitro one, or partial peptide of Cryj2, and increases. On the other hand with the mouse which derived immunological tolerance with peptide of this invention beforehand. Even if it performs immunity by the peptide 13 after that, it is not activated, and a T cell specific to the peptide does not react to the peptide 13 shown by the antigen presenting cell in in vitro one, or partial peptide of Cryj2, and is not increased. By measuring the difference, the curative effect over the hay fever of peptide of this invention can be checked.

[0049]Peptide of this invention can inactivate a specific T cell to cedar pollen allergen, without causing anaphylaxis substantially, if the general mammals including Homo sapiens are medicated since it does not react to a specific immunoglobulin E antibody substantially at cedar pollen allergen.

[0050]The anti-hay fever agent of this invention which contains peptide of this invention, its complex, its derivative, or its polymer as an active principle can treat hay fever, without causing side effects, such as anaphylaxis, substantially, if the patient suffered from hay fever is medicated. When, medicating a healthy individual and the individual of potential hay fever with the anti-hay fever agent of this invention on the other hand before cedar pollen begins to disperse, while demonstrating a remarkable preventive effect to hay fever, higher efficacy is demonstrated to remission of the allergies at the time of the onset.

[0051]When it explains to the anti-hay fever agent per pan of this invention in detail, the anti-hay fever agent of this invention usually, One sort of peptide of this invention, its complex, its derivative, or its polymer or two sorts or more are included 0.5 thru/or 5.0% (w/w) still more preferably 0.05 thru/or 50% (w/w) preferably 0.01 thru/or 100% (w/w). . As for the anti-hay fever agent of this invention, a gestalt peptide independent [concerned] is permitted physiologically in addition to it from the first. For example, serum albumin, gelatin, glucose, a shook sirloin, Lactose, malt sugar, trehalose, sorbitol, maltitol, Carriers, such as a RAKUCHI toll, mannitol, and pullulan, an excipient, an immunoadjuvant, The gestalt as a constituent combined with stabilizer, one sort which contains anti-inflammatory agents, such as steroid hormone and disodium cromoglycate, an antihistamine, an anti leukotriene agent, and an anti-tachykinin agent if needed further, or two sorts or more of other drugs is included. The anti-hay fever agent of this invention also includes the drugs of medication unit form voice, and with the drugs of the medication unit form voice. The quantity which is equivalent to the dosage per day, its integral multiple (up to 4 times), or its divisor, for example in peptide of this invention, its complex, its derivative, or its polymer (to 1/40) is contained, and the drugs in the dosage forms of one suitable for administration separated physically are meant. As drugs of such medication unit form voice, powder medicine, subtle granules, a granule, a pill, a tablet, a capsule, the trochiscus,

syrups, an emulsion, an ointment, plaster, cataplasms, suppositories, ophthalmic solutions, a nasal drop, a spray, injections, etc. are mentioned.

[0052]the anti-hay fever agent of this invention -- a purpose [of hay fever / the therapy or prevention] -- taking orally, transderma, and the rhinenchysis -- a medicine is applied eyewash or injection prescribed for the patient Although the dosage in Homo sapiens changes also according to the purpose of administration, a method, and condition, usually -- while observing a candidate's condition and the progress after administration -- an adult -- 0.01 thru/or 1000 mg per day is 1 time of frequency about 1 thru/or 10 mg preferably 1 time thru/or every month every day at a rule of thumb -- about -- repeated-dose administration is usually carried out for 1 thru/or 6 months, increasing a dosage.

[0053]

[Example]Hereafter, although an example explains this invention still in detail, as for this invention, the technical scope is not limited by these.

[Example 1] Chemosynthesis of the peptide was carried out by the method (solid-phase-synthesis method) of combining amino acid at a time with one amino acid derivative fixed to peptide 1 resin from one end in the end of carboxyl. For the purpose of checking a synthetic situation, and the purpose of adding a reagent to a synthetic machine, composition of the peptide 1 (array number 1 of an array table) was performed by dividing into five steps so that it might indicate below.

[0054]1) It was thought that composition of long chain peptide would become possible by lessening the rate of the peptide chain compounded on resin. Then, a part of alpha-amino group of the C-terminal amino acid beforehand introduced into the resin used for solid phase synthesis was blocked by the acetyl group, the rate of the peptide compounded on resin was reduced, and composition of long chain peptide was tried.

[0055]The amino acid used in each cycle used the special amino acid derivative with which alpha-amino group and the reaction group of the residue portion were blocked by the protective group. For example, alpha-amino group of each amino acid derivative is blocked by Fmoc (9-fluorenyl MECHIROKISHI carbonyl). Peptide synthesis carried out deprotection of the Fmoc of alpha-amino group of the amino acid combined with resin, repeated successively the reaction of combining the amino acid derivative which the carboxyl group activated next, and was performed (the Fmoc method).

[0056]Since it is known that a peptide chain will be easily missing from resin if the Wang resin used widely by the Fmoc method is used for composition of the peptide which has Pro in a C terminal, As resin for the solid-phase-synthesis methods of the peptide 1, 2-chloro trityl (2-Cl-Trt) resin (henceforth "Cl-Trt resin") was used.

[0057]An equivalent for 100micromol of the Pro-Cl-Trt resin in which the amino acid (Pro) equivalent to the C terminal residue of the peptide 1 is introduced is put into the conical tube made from 15-ml ** polypropylene, 5 ml of CAP liquid (dimethylformamide: N-methylmorpholine : acetic anhydride =520:1:1 (volume ratio)) containing the acetic anhydride of 96micromol was added, and it settled for 30 minutes at the room temperature. A part of alpha-amino group of the C-terminal amino acid of a large number introduced into resin is blocked by an acetyl group by this operation, and since next peptide synthesis advances only from alpha-amino group which escaped the block, the maximum number of the peptide compounded on resin decreases.

[0058]this resin -- a peptide synthesis machine and a symphony (made by Aloka) -- it putting into ***** SSERU made from a disposable plastic of business, and, After dichloromethane washed resin, it moved to ***** SSERU of a peptide synthesis machine and model 430A (made by an applied bio-systems company) business, and compounded in accordance with the Fmoc solid-phase-synthesis method (the fast mock method of the device (FastMoc Chemistry)) using this synthetic machine. To Fmoc-Asp(OtBu) 1mmol which is a derivative of the 2nd amino acid, from the end side of C first. Activator solution [2M. Diisopropylethylamine. (DIEA) /N-methyl pyrrolidone. (It is called the following "NMP") 1 ml and 0.45M. 2.2 ml of 2-(1H-benzotriazol 1-yl)-1,1,3,3-tetramethyl RONIUMU hexafluorophosphate (HBTU) / 1-hydroxybenzotriazol (HOBt) / dimethylformamide, and NMP 1ml]. In addition, at that time, although the activated thing (it is 10 time equivalent weight to joint amino acid) and the resin blocked by the previous acetyl group

were made to react at a room temperature, the double cup Ling's method which repeats this reaction twice was used in order to fully combine amino acid. Since it was hard to combine amino acid with Pro-Cl-Trt resin at this time, reaction time was made into 120 minutes (usually 30 minutes).

[0059]The Fmoc-Asp(OtBu)-Pro-Cl-Trt resin generated here by NMP 7ml After 7 times washing, 7 ml of deprotection solutions (piperidine/NMP) were made to react twice for 3 minutes and for 12 minutes, and 7 ml of NMP liquid washed 5 times except for the Fmoc basis of the amino acid combined with resin. Next, the double cup ring reaction of the activation Fmoc-Tyr(tBu) solution which is a derivative of the 3rd amino acid was carried out from the end side of C. Hereafter, the same operation was repeated. However, the piperidine concentration of the deprotection solution of a Fmoc basis was gradually raised according to the length of the peptide compounded from 23% (1 cycle eye) to 36% (20 cycle eye).

[0060]It is the above-mentioned method and is peptide: Fmoc-Lys(Boc)-Lys(Boc)-Glu(OtBu)-Ala-Phe-Asn(Trt)-Val-Glu(OtBu)-Tyr(tBu)-Gly-Leu-Val-His(Trt)-Val- from a C terminal to 21 residue eye. When Ala-Asn(Trt)-Asn(Trt)-Asn(Trt)-Tyr(tBu)-Asp(OtBu)-Pro-Cl-Trt resin was compounded, composition was interrupted after the deFmoc reaction. 1 mg of the above-mentioned resin is extracted, and it is 6N. After chloride performed hydrolysis at 110 ** for 24 hours, the separated amino acid was measured with the amino acid analyzer (L-8500 type, the Hitachi [, Ltd.], Ltd. make). As a result, it became clear that the peptide combined with resin was about 20% of the amount of resin substitution.

[0061]Furthermore, extract resin in part, add a 0.5-ml KURIBEJI solution (ethanedithiol: triphloroacetic acid (henceforth "TFA") : water : triisopropyl silane =92.5:2.5:2.5:2.5) to resin, and a KURIBEJI reaction is performed for 1 hour, Ether was added to the obtained peptide solution and peptide was settled [1997/98 peptide-synthesis handbook S 48; Novabiochem]. using a mass spectrometer (made in [VG biotech company] VG plat form) about the obtained peptide -- electrospray -- Io -- NAIZESHON (electrospray ionisation and henceforth "ESI") -- the molecular weight was measured in law and the synthetic product was checked.

[0062]2) Peptide synthesis is succeedingly performed using the remaining resin of the above 1, Peptide from a C terminal to 31 residue eye: Fmoc-Gly-Lys(Boc)-Tyr(tBu)-Glu(OtBu)-Gly-Gly-Asn(Trt)-Ile-Tyr(tBu)-Thr(tBu)-Lys(Boc)-Lys(Boc)-Glu(OtBu) -Ala-Phe-Asn(Trt)-Val-Glu(OtBu)-Tyr(tBu)-Gly-Leu-Val-His(Trt)-Val-Ala-Asn(Trt)-Asn(Trt)-Asn(Trt)-Tyr(tBu)-Asp(OtBu)-Pro-Cl. - When Trt resin was compounded, the composition after a deFmoc reaction was interrupted. Resin was taken out in part, the KURIBEJI reaction was performed, and the synthetic product was checked by the same method as the above 1.

[0063]3) Peptide synthesis is succeedingly performed using the remaining resin of the above 2, Peptide from a C terminal to 51 residue eye : Fmoc-Phe-His(Trt)-Leu-Gln(Trt)-Lys(Boc)-Asn(Trt)-Thr(tBu)-Lys(Boc)-Leu-Thr(tBu)-Ser. (tBu)-G ly-Lys(Boc)-Ile-Ala-Ser(tBu)-Cys(Trt)-Leu-Asn(Trt)-Ser(tBu)-Gly-Lys(Boc)-Tyr(tBu)-Glu(OtBu)-Gly-Gly-Asn(Trt) -Ile-Tyr(tBu)-Thr(tBu)-Lys(Boc)-Lys(Boc)-Glu(OtBu)-Ala-Phe-Asn(Trt)-Val-Glu(OtBu)-Tyr(tBu)-Gly-Leu-Val-His(Trt)-Val-Ala-. When Asn(Trt)-Asn(Trt)-Asn(Trt)-Tyr(tBu)-Asp(OtBu)-Pro-Cl-Trt resin was compounded, the composition after a deFmoc reaction was interrupted. Resin was taken out in part, the KURIBEJI reaction was performed, and the synthetic product was checked by the same method as the above 1.

[0064]4) Peptide synthesis is succeedingly performed using the remaining resin of the above 3, Peptide from a C terminal to 71 residue eye: Fmoc-Ser(tBu)-Trp(Boc)-Ser(tBu)-Met-Lys(Boc)-Val-Thr(tBu)-Val-Ala-Phe-Asn(Trt)-Gln(Trt)-Phe-Gly-Pro-Phe- Ala-Ser (tBu) -Lys(Boc)-Asn(Trt)-Phe-His(Trt)-Leu-Gln(Trt)-Lys(Boc)-Asn(Trt)-Thr(tBu)-Lys(Boc)-Leu-Thr(tBu)-Ser(tBu) -Gly-Lys(Boc)-Ile-Ala-Ser(tBu)-Cys(Trt)-Leu-Asn(Trt)-Ser(tBu)-Gly-Lys(Boc)-Tyr(tBu)-Glu(OtBu)-Gly-Gly-Asn(Trt) -Ile-Tyr(tBu)-Thr(tBu)-Lys(Boc)-Lys(Boc)-Glu(OtBu)-Ala-Phe-Asn(Trt)-Val-Glu(OtBu)-Tyr(tBu)-Gly-Leu-Val-His(Trt)-Val-Ala-. When Asn(Trt)-Asn(Trt)-Asn(Trt)-Tyr(tBu)-Asp(OtBu)-Pro-Cl-Trt resin was compounded, composition was interrupted after the deFmoc reaction. The reaction which combines Fmoc-Ser (tBu) of 69 residue eye, Fmoc-Trp (Boc) of 70 residue eye, and Fmoc-Ser (tBu) of 71 residue eye from a C terminal, Since it was predicted that reaction efficiency is not good, the reaction by a deprotection solution was

repeated further 3 times, and after making it the Fmoc basis combined with peptide fully separate, reaction time which combines amino acid was carried out in 120 minutes, and was performed. Resin was extracted in part, the KURIBEJI reaction was performed, and the synthetic product was checked by the same method as the above 1.

[0065]5) Peptide synthesis is succeedingly performed using the remaining resin of the above 4, Peptide from a C terminal to 81 residue eye: Fmoc-Gly-Ile-Ile-Ala-Ala-Tyr(tBu)-Gln(Trt)-Asn(Trt)-Pro-Ala-Ser(tBu)-Trp(Boc)-Ser(tBu)-Met-Lys(Boc)-Val-Thr(tBu)-Val-Ala-Phe-Asn(Trt)-Gln(Trt)-Phe-Gly-Pro-Phe-Ala-Ser(tBu)-Lys(Boc)-Asn(Trt)-Phe-His(Trt)-Leu-Gln(Trt)-Lys(Boc)-Asn(Trt)-Thr(tBu)-Lys(Boc)-Leu-Thr(tBu)-Ser(tBu)-Gly-Lys(Boc)-Ile-Ala-Ser(tBu)-Cys(Trt)-Leu-Asn(Trt)-Ser(tBu)-Gly-Lys(Boc)-Tyr(tBu)-Glu(OtBu)-Gly-Gly-Asn(Trt)-Ile-Tyr(tBu)-Thr(tBu)-Lys(Boc)-Lys(Boc)-Glu(OtBu)-Ala-Phe-Asn(Trt)-Val-Glu(OtBu)-Tyr(tBu)-Gly-Leu-Val-His(Trt)-Val-Ala-Asn(Trt)-Asn(Trt)-Asn(Trt)-Tyr(tBu)-Asp(OtBu)-Pro-Cl- - Trt resin was compounded.

[0066]:Fmoc-Ala which is as follows [amino acid derivative / which was used for composition] here, Fmoc-Arg (Pmc), Fmoc-Asn (Trt), Fmoc-Asp (OtBu), Fmoc-Cys (Trt), Fmoc-Gln (Trt), Fmoc-Glu (OtBu), Fmoc-Gly, Fmoc-His (Trt), Fmoc-Ile, Fmoc-Leu, Fmoc-Lys (Boc), Fmoc-Met, Fmoc-Phe, Fmoc-Pro, Fmoc-Ser (tBu), Fmoc-Thr (tBu), Fmoc-Trp (Boc), Fmoc-Tyr (tBu), Fmoc-Val (; showing the protective group from which the inside of () protects the reaction group of a residue portion product made from a PerkinElmer Japan Co., Ltd. applied bio-systems operation division).

[0067]The deprotection solution was made to react to the protection peptide resin produced by performing it above, and deprotection of the Fmoc basis of an amino terminal was carried out. Next, dichloromethane washed 3 times after 6 times washing in 7-ml NMP, and it was made to dry for 15 minutes by spraying argon gas further. By taking out resin, adding 30 ml of KURIBEJI solutions and making them react at a room temperature for 2 hours, cutting of peptide from resin and removal of the amino acid side chain protective group were performed, and the peptide solution was obtained.

[0068]This peptide solution was filtered using the poly TETORARU fluoroethylene (henceforth "PTFE") filter (3.0 micrometers, ADVANTEC Oriental incorporated company make), and filtrate was collected to the centrifuging tube. After condensing this using an aspirator, 80 ml of cold ether was added and peptide was settled. Precipitate was washed by repeating the operation which centrifuges this thing, collects precipitate (for 3000 rpm and 10 minutes) after cooling for a while, and are collected if cold ether is added again and it is made to distribute 3 times. The obtained precipitate was dried and 252 mg of rough peptide was obtained.

[0069]After dissolving 75 mg of this rough peptide in the 20% acetonitrile solution which contains TFA 0.1%, : with which high performance chromatography (henceforth "HPLC") was presented on condition of the following -- column: -- an ODS column (TSK-gel ODS-120T, the particle diameter of 5 micrometers, 120 A in an aperture, column size phi21.5mmx300mm, TOSOH [CORP.] CORP. make)

Mobile phase: 35-37% acetonitrile / 0.1% TFA, 30 minutes (linear density gradient)

rate-of-flow: -- a part for 5-ml/-- detection wave length: -- the fraction eluted in 220-nm 23 to 25 minutes was isolated preparatively, freeze-drying was performed after concentration, and 11.5 mg of target peptide was obtained.

[0070]the peptide obtained here still contained the impurity -- a sake -- :column:C22 column (the docosyl- B, column size phi10mmx250mm, made in Seng Shue) of further the following which performed HPLC refining on conditions

Mobile phase: 30-31% acetonitrile / 0.1% TFA, 30 minutes (linear density gradient)

rate-of-flow: -- a part for 1.5-ml/-- detection wave length: -- the fraction eluted in 220-nm 32 to 34 minutes was isolated preparatively, freeze-drying was performed after concentration, and 2.5 mg of target peptide was obtained.

[0071]About this refining peptide, the molecular weight was checked by the ESI method using the mass spectrometer. further -- this peptide 100pmol -- an amino acid sequence analyzer (PPSQ-10 type.) As a result of conducting amino acid sequence analysis from an amino terminal to 10 residue using the Shimadzu [Corp.] Corp. make, it was checked that it is in agreement

with the amino acid sequence shown in the amino acid numbers 1-10 among the array numbers 1 of an array table.

[0072][Example 2] For the purpose of checking a synthetic situation, and the purpose of adding a reagent to a synthetic machine, composition of the peptide 2 peptide 2 (array number 2 of an array table) was performed by dividing into four steps so that it might indicate below.

1) It was desorbed from the Fmoc basis by adding 6 ml of 20% piperidine to an equivalent for 100micromol of the Fmoc-Glu(OtBu)-Wang resin in which the amino acid (Glu) equivalent to the C terminal residue of the peptide 2 is introduced, and making it react to it at a room temperature for 30 minutes. 5 ml of CAP liquid was made to react for 30 minutes at a room temperature after washing resin. About 80% of alpha-amino groups of the C-terminal amino acid introduced into resin is blocked by an acetyl group like Example 1 by this operation, and since next peptide synthesis advances only from alpha-amino group which escaped the block, the maximum number of the peptide compounded on resin decreases.

[0073]Move to ***** SSERU after washing resin, and first, Peptide from a C terminal to 21 residue eye: Fmoc-Asp(OtBu)-Pro-Ser(tBu)-Gly-Lys(Boc)-Tyr(tBu)-Glu(OtBu)-Gly-Gly-Asn(Trt)-Ile-Tyr(tBu)-Thr(tBu)-Lys(When Boc-Lys(Boc)-Glu(OtBu)-Ala-Phe-Asn(Trt)-Val-Glu(OtBu)-Wang resin was compounded, composition was interrupted after the deFmoc reaction. Resin was taken out in part, the KURIBEJI reaction was performed, and the synthetic product was checked by the method indicated to 1 of Example 1.

[0074]2) Peptide synthesis is succeedingly performed using the remaining resin of the above 1, Peptide from a C terminal to 41 residue eye: Fmoc-Ser(tBu)-Gly-Lys(Boc)-Ile-Ala-Ser(tBu)-Cys(Trt)-Leu-Asn(Trt)-Tyr(tBu)-Gly-Leu-Val-His(Trt)-Val-Ala- Asn (Trt) -Asn(Trt)-Asn(Trt)-Tyr(tBu)-Asp(OtBu)-Pro-Ser(tBu)-Gly-Lys(Boc)-Tyr(tBu)-Glu(OtBu)-Gly-Gly-Asn(Trt)-Ile-Tyr(tBu)-Thr(tBu)-. When Lys(Boc)-Lys(Boc)-Glu(OtBu)-Ala-Phe-Asn(Trt)-Val-Glu(OtBu)-Wang resin was compounded, composition was interrupted after the deFmoc reaction. Resin was taken out in part, the KURIBEJI reaction was performed, and the synthetic product was checked by the method indicated to 1 of Example 1.

[0075]3) Peptide synthesis is succeedingly performed using the remaining resin of the above 2, Peptide from a C terminal to 61 residue eye : Fmoc-Phe-Asn(Trt)-Gln(Trt)-Phe-Gly-Phe-Ala-Ser(tBu)-Lys(Boc)-Asn(Trt)-Phe-His(Trt)-Leu-Gln(Trt)-Lys(Boc) -Asn(Trt)-Thr(tBu)-Lys(Boc)-Leu-Thr(tBu)-Ser(tBu)-Gly-Lys(Boc)-Ile-Ala-Ser(tBu)-Cys(Trt)-Leu-Asn(Trt)-Tyr(tBu)-Gly-Leu-Val. - His. (Trt)-Val-Ala-Asn. (Trt)-Asn. (Trt)-Asn. (Trt)-Tyr. (tBu)-Asp. (OtBu) -Pro-Ser(tBu)-Gly-Lys(Boc)-Tyr(tBu)-Glu(OtBu)-Gly-Gly-Asn(Trt)-Ile-Tyr(tBu)-Thr(tBu)-Lys(Boc)-Lys(Boc)-Glu(OtBu)-Ala. - When Phe-Asn(Trt)-Val-Glu(OtBu)-Wang resin was compounded, composition was interrupted after the deFmoc reaction. Resin was taken out in part, the KURIBEJI reaction was performed, and the synthetic product was checked by the method indicated to 1 of Example 1.

[0076]4) Peptide synthesis was succeedingly performed using the remaining resin of the above 3, and peptide from a C terminal to 79 residue eye was compounded. However, the reaction which combines Fmoc-Trp (Boc) of 68 residue eye, and Fmoc-Ser (tBu) of 69 residue eye from a C terminal, Since it was predicted that reaction efficiency is not good, the reaction by a deprotection solution was repeated further 3 times, and after making it the Fmoc basis combined with peptide fully separate, the reaction time which combines amino acid was extended in 120 minutes, and was performed.

[0077]By the above method, it is Fmoc-Gly-Ile-Ile-Ala-Ala-Tyr(tBu)-Gln(Trt)-Asn(Trt)-Pro-Ala-Ser(tBu)-Trp(Boc)-Met-Lys(Boc)-Val-Thr(tBu). -Val-Ala-Phe-Asn(Trt)-Gln(Trt)-Phe-Gly-Phe-Ala-Ser(tBu)-Lys(Boc)-Asn(Trt)-Phe-His(Trt)-Leu-Gln(Trt)-Lys(Boc) -Asn(Trt)-Thr(tBu)-Lys(Boc)-Leu-Thr(tBu)-Ser(tBu)-Gly-Lys(Boc)-Ile-Ala-Ser(tBu)-Cys(Trt)-Leu-Asn(Trt) -Tyr(tBu)-Gly-Leu-Val-His(Trt)-Val-Ala-Asn(Trt)-Asn(Trt)-Asn(Trt)-Tyr(tBu)-Asp(OtBu)-Pro-Ser(tBu)-Gly-Lys(Boc) -Tyr(tBu)-Glu(OtBu)-Gly-Gly-Asn(Trt)-Ile-Tyr(tBu)-Thr(tBu)-Lys(Boc)-Lys(Boc)-Glu(OtBu)-Ala-Phe-Asn(Trt)-Val-Glu(OtBu)-Wang. Resin was obtained. The deFmoc reaction and the KURIBEJI reaction were performed to this thing, and 194 mg of rough peptide was obtained.

[0078]:column which performed HPLC refining on condition of the following after dissolving 97

mg of this rough peptide in the 20% acetonitrile solution which contains TFA 0.1%: ODS column (TSK-gel ODS-120T)

Mobile phase: 35–38% acetonitrile / 0.1% TFA, 30 minutes (linear density gradient)

rate-of-flow: -- a part for 5-ml/-- detection wave length: -- the fraction eluted in 220-nm 27 to 28 minutes was isolated preparatively, freeze-drying was performed after concentration, and 7 mg of target peptide was obtained.

[0079]:column:C22 column which performed HPLC refining further on condition of the following since the peptide obtained here still contained the impurity (docosyl- B)

Mobile phase: 31–32% acetonitrile / 0.1% TFA, 30 minutes (linear density gradient)

rate-of-flow: -- a part for 1.5-ml/-- detection wave length: -- the fraction eluted in 220-nm 20 to 22 minutes was isolated preparatively, freeze-drying was performed after concentration, and 2.3 mg of target peptide was obtained.

[0080]About this refining peptide, the molecular weight was checked by the ESI method using the mass spectrometer. About refining peptide 100pmol, as a result of conducting amino acid sequence analysis from an amino terminal to 10 residue, it was checked that it is in agreement with the amino acid sequence shown in the amino acid numbers 1–10 among the array numbers 2 of an array table.

[0081][Example 3] For the purpose of checking a synthetic situation, and the purpose of adding a reagent to a synthetic machine, composition of the peptide 3 peptide 3 (array number 3 of an array table) was performed by dividing into five steps so that it might indicate below.

1) First by the method indicated in the Example 1. Peptide from a C terminal to 21 residue eye: Fmoc-Lys(Boc)-Lys(Boc)-Glu(OtBu)-Ala-Phe-Asn(Trt)-Val-Glu(OtBu)-Tyr(tBu)-Gly-Leu-Val-His(Trt)-Val-Ala-Asn (Trt) When -Asn(Trt)-Asn(Trt)-Tyr(tBu)-Asp(OtBu)-Pro-Cl-Trt resin was compounded, composition was interrupted after the deFmoc reaction. Resin was extracted in part, the KURIBEJI reaction was performed, and the synthetic product was checked by the method indicated to 1 of Example 1.

[0082]2) Peptide synthesis is succeedingly performed using the remaining resin of the above 1, Peptide from a C terminal to 41 residue eye: Fmoc-Ser(tBu)-Gly-Lys(Boc)-Ile-Ala-Ser(tBu)-Cys(Trt)-Leu-Asn(Trt)-Ser(tBu)-Gly-Lys(Boc)-Tyr(tBu)-Glu(OtBu)-Gly-Gly-Asn(Trt)-Ile-Tyr(tBu)-Thr(tBu)-Lys(Boc)-Lys(Boc)-Glu(OtBu)-Ala-Phe-Asn(Trt)-Val-Glu(OtBu)-Tyr(tBu)-Gly-Leu-Val-. When His(Trt)-Val-Ala-Asn(Trt)-Asn(Trt)-Asn(Trt)-Tyr(tBu)-Asp(OtBu)-Pro-Cl-Trt resin was compounded, composition was interrupted after the deFmoc reaction. Resin was extracted in part, the KURIBEJI reaction was performed, and the synthetic product was checked by the method indicated to 1 of Example 1.

[0083]3) Peptide synthesis is succeedingly performed using the remaining resin of the above 2, Peptide from a C terminal to 51 residue eye: Fmoc-Phe-His(Trt)-Leu-Gln(Trt)-Lys(Boc)-Asn(Trt)-Thr(tBu)-Lys(Boc)-Leu-Thr(tBu)-Ser(tBu)-Gly-Lys(Boc)-Ile-Ala-Ser(tBu)-Cys(Trt)-Leu-Asn(Trt)-Ser(tBu)-Gly-Lys(Boc)-Tyr(tBu)-Glu(OtBu)-Gly-Gly-Asn(Trt)-Ile-Tyr(tBu)-Thr(tBu)-Lys(Boc)-Lys(Boc)-Glu(OtBu)-Ala-Phe-Asn(Trt)-Val-Glu(OtBu)-Tyr(tBu)-Gly-Leu-Val-His(Trt)-Val-Ala-Asn(Trt)-Asn(.) When Trt-Asn(Trt)-Tyr(tBu)-Asp(OtBu)-Pro-Cl-Trt resin was compounded, composition was interrupted after the deFmoc reaction. Resin was extracted in part, the KURIBEJI reaction was performed, and the synthetic product was checked by the method indicated to 1 of Example 1.

[0084]4) Peptide synthesis is succeedingly performed using the remaining resin of the above 3, Peptide of up to [from a C terminal] 61 residue : Fmoc-Phe-Asn(Trt)-Gln(Trt)-Phe-Gly-Phe-Ala-Ser(tBu)-Lys(Boc)-Asn(Trt)-Phe-His(Trt)-Leu-Gln(Trt)-Lys(Boc). - Asn. (Trt)-Thr. (tBu)-Lys. (Boc)-Leu-Thr(tBu)-Ser(tBu)-Gly-Lys(Boc)-Ile-Ala-Ser(tBu)-Cys(Trt)-Leu-Asn(Trt)-Ser(tBu)-Gly-Lys(Boc)-Tyr(tBu)-Glu(OtBu)-Gly-Gly-Asn(Trt)-Ile-Tyr(tBu)-Thr(tBu)-Lys(Boc)-Lys(Boc)-Glu(OtBu)-Ala-Phe-Asn(Trt)-Val-Glu(OtBu)-Tyr(tBu)-Gly-Leu-Val-. When His(Trt)-Val-Ala-Asn(Trt)-Asn(Trt)-Asn(Trt)-Tyr(tBu)-Asp(OtBu)-Pro-Cl-Trt resin was compounded, composition was interrupted after the deFmoc reaction. Resin was extracted in part, the KURIBEJI reaction was performed, and the synthetic product was checked by the method indicated to 1 of Example 1.

[0085]5) Peptide synthesis was succeedingly performed using the remaining resin of the above 4,

and peptide from a C terminal to 79 residue eye was compounded. However, the reaction which combines Fmoc-Trp (Boc) of 68 residue eye, and Fmoc-Ser (tBu) of 69 residue eye from a C terminal, Since it was predicted that reaction efficiency is not good, the reaction by a deprotection solution was repeated further 3 times, and after making it the Fmoc basis combined with peptide fully separate, the reaction time which combines amino acid was extended in 120 minutes, and was performed.

[0086] By the above method, it is Fmoc-Gly-Ile-Ile-Ala-Ala-Tyr(tBu)-Gln(Trt)-Asn(Trt)-Pro-Ala-Ser(tBu)-Trp(Boc)-Met-Lys(Boc)-Val-Thr(tBu). -Val-Ala-Phe-Asn(Trt)-Gln(Trt)-Phe-Gly-Phe-Ala-Ser(tBu)-Lys(Boc)-Asn(Trt)-Phe-His(Trt)-Leu-Gln(Trt)-Lys(Boc) -Asn(Trt)-Thr(tBu)-Lys(Boc)-Leu-Thr(tBu)-Ser(tBu)-Gly-Lys(Boc)-Ile-Ala-Ser(tBu)-Cys(Trt)-Leu-Asn(Trt) -Ser(tBu)-Gly-Lys(Boc)-Tyr(tBu)-Glu(OtBu)-Gly-Gly-Asn(Trt)-Ile-Tyr(tBu)-Thr(tBu)-Lys(Boc)-Lys(Boc)-Glu(OtBu) -Ala-Phe-Asn(Trt)-Val-Glu(OtBu)-Tyr(tBu)-Gly-Leu-Val-His(Trt)-Val-Ala-Asn(Trt)-Asn(Trt)-Asn(Trt)-Tyr(tBu)-Asp(OtBu)-Pro-Cl. - Trt resin was obtained. The deFmoc reaction and the KURIBEJI reaction were performed to this thing, and 380 mg of rough peptide was obtained. among these, :column:ODS column (TSK-gel ODS-120T) which carried out HPLC refining on condition of the following after dissolving 190 mg in the 20% acetonitrile solution which contains TFA 0.1%

Mobile phase: 35-37% acetonitrile / 0.1% TFA, 30 minutes (linear density gradient)

rate-of-flow: -- a part for 5-ml/-- detection wave length: -- the fraction eluted in 220-nm 25 to 27 minutes was isolated preparatively, freeze-drying was performed after concentration, and 15.6 mg of target peptide was obtained.

[0087] About this refining peptide, the molecular weight was checked by the ESI method using the mass spectrometer. About refining peptide 100pmol, as a result of conducting amino acid sequence analysis from an amino terminal to 10 residue, it was checked that it is in agreement with the amino acid sequence shown in the amino acid numbers 1-10 among the array numbers 3 of an array table.

[0088][Example 4] For the purpose of checking a synthetic situation, and the purpose of adding a reagent to a synthetic machine, composition of the peptide 4 peptide 4 (array number 4 of an array table) was performed by dividing into five steps so that it might indicate below.

1) First by the method indicated in the Example 2. Peptide from a C terminal to 21 residue eye: Fmoc-Asp(OtBu)-Pro-Ser(tBu)-Gly-Lys(Boc)-Tyr(tBu)-Glu(OtBu)-Gly-Gly-Asn(Trt)-Ile-Tyr(tBu)-Thr(tBu)-Lys(When Boc-Lys(Boc)-Glu(OtBu)-Ala-Phe-Asn(Trt)-Val-Glu(OtBu)-Wang resin was compounded, composition was interrupted after the deFmoc reaction. Resin was extracted in part, the KURIBEJI reaction was performed, and the synthetic product was checked by the method indicated to 1 of Example 1.

[0089] 2) Peptide synthesis is succeedingly performed using the remaining resin of the above 1, Peptide of up to [from a C terminal] 41 residue: Fmoc-Ser(tBu)-Gly-Lys(Boc)-Ile-Ala-Ser(tBu)-Cys(Trt)-Leu-Asn(Trt)-Tyr(tBu)-Gly-Leu-Val-His(Trt)-Val-Ala- Asn (Trt) -Asn(Trt)-Asn(Trt)-Tyr(tBu)-Asp(OtBu)-Pro-Ser(tBu)-Gly-Lys(Boc)-Tyr(tBu)-Glu(OtBu)-Gly-Gly-Asn(Trt)-Ile-Tyr(tBu)-Thr(tBu)-. When Lys(Boc)-Lys(Boc)-Glu(OtBu)-Ala-Phe-Asn(Trt)-Val-Glu(OtBu)-Wang resin was compounded, composition was interrupted after the deFmoc reaction. Resin was extracted in part, the KURIBEJI reaction was performed, and the synthetic product was checked by the method indicated to 1 of Example 1.

[0090] 3) Peptide synthesis is succeedingly performed using the remaining resin of the above 2, Peptide from a C terminal to 61 residue eye : Fmoc-Asn(Trt)-Gln(Trt)-Phe-Gly-Pro-Phe-Ala-Ser(tBu)-Lys(Boc)-Asn(Trt)-Phe-His(Trt)-Leu-Gln(Trt)-Lys(Boc) -Asn(Trt)-Thr(tBu)-Lys(Boc)-Leu-Thr(tBu)-Ser(tBu)-Gly-Lys(Boc)-Ile-Ala-Ser(tBu)-Cys(Trt)-Leu-Asn(Trt) -Tyr(tBu)-Gly-Leu-Val-His(Trt)-Val-Ala-Asn(Trt)-Asn(Trt)-Asn(Trt)-Tyr(tBu)-Asp(OtBu)-Pro-Ser(tBu)-Gly-Lys(Boc) -Tyr(tBu)-Glu(OtBu)-Gly-Gly-Asn(Trt)-Ile-Tyr(tBu)-Thr(tBu)-Lys(Boc)-Lys(Boc)-Glu(OtBu)-Ala-Phe-Asn(Trt)-Val-Glu(OtBu)-Wang. When resin was compounded, composition was interrupted after the deFmoc reaction. Resin was extracted in part, the KURIBEJI reaction was performed, and the synthetic product was checked by the method indicated to 1 of Example 1.

[0091] 4) Peptide synthesis is succeedingly performed using the remaining resin of the above 3,

Peptide from a C terminal to 81 residue eye: Fmoc-Gly-Ile-Ile-Ala-Ala-Tyr(tBu)-Gln(Trt)-Asn(Trt)-Pro-Ala-Ser(tBu)-Trp(Boc)-Ser(tBu)-Met-Lys(Boc)-Val-Thr(tBu)-Val-Ala-Phe-Asn(Trt)-Gln(Trt)-Phe-Gly-Pro-Phe-Ala-Ser(tBu)-Lys(Boc)-Asn(Trt)-Phe-His(Trt)-Leu-Gln(Trt)-Lys(Boc)-Asn(Trt)-Thr(tBu)-Lys(Boc)-Leu-Thr(tBu)-Ser(tBu)-Gly-Lys(Boc)-Ile-Ala-Ser(tBu)-Cys(Trt)-Leu-Asn(Trt)-Tyr(tBu)-Gly-Leu-Val-His(Trt)-Val-Ala-Asn(Trt)-Asn(Trt)-Asn(Trt)-Tyr(tBu)-Asp(OtBu)-Pro-Ser(tBu)-Gly-Lys(Boc)-Tyr(tBu)-Glu(OtBu)-Gly-Gly-Asn. When (Trt)-Ile-Tyr(tBu)-Thr(tBu)-Lys(Boc)-Lys(Boc)-Glu(OtBu)-Ala-Phe-Asn(Trt)-Val-Glu(OtBu)-Wang resin was compounded, composition was interrupted after the deFmoc reaction. However, the reaction which combines Fmoc-Ser(tBu) of 69 residue eye, Fmoc-Trp(Boc) of 70 residue eye, and Fmoc-Ser(tBu) of 71 residue eye from a C terminal, Since it was predicted that reaction efficiency is not good, the reaction by a deprotection solution was repeated further 3 times, and after making it the Fmoc basis combined with peptide fully separate, the reaction time which combines amino acid was extended in 120 minutes, and was performed. Resin was extracted in part, the KURIBEJI reaction was performed, and the synthetic product was checked by the method indicated to 1 of Example 1.

5) Peptide synthesis was succeedingly performed using the remaining resin of the above 4, and peptide from a C terminal to 93 residue eye was compounded.

[0092] By the above method, it is Fmoc-Gln(Trt)-Phe-Ala-Lys(Boc)-Leu-Thr(tBu)-Gly-Phe-Thr(tBu)-Leu-Met-Gly-Gly-Ile-Ile-Ala-Ala-Tyr(tBu)-Gln(Trt)-Asn(Trt)-Pro-Ala-Ser(tBu)-Trp(Boc)-Ser(tBu)-Met-Lys(Boc)-Val-Thr(tBu)-Val-Ala-Phe-Asn(Trt)-Gln(Trt)-Phe-Gly-Pro-Phe-Ala-Ser(tBu)-Lys(Boc)-Asn(Trt)-Phe-His(Trt)-Leu-Gln(Trt)-Lys(Boc)-Asn(Trt)-Thr(tBu)-Lys(Boc)-Leu-Thr(tBu)-Ser(tBu)-Gly-Lys(Boc)-Ile-Ala-Ser(tBu)-Cys(Trt)-Leu-Asn(Trt)-Tyr(tBu)-Gly-Leu-Val-His(Trt)-Val-Ala-Asn(Trt)-Asn(Trt)-Asn(Trt)-Tyr(tBu)-Asp(OtBu)-Pro-Ser(tBu)-Gly-Lys(Boc)-Tyr(tBu)-Glu(OtBu)-Gly-Gly-Asn(Trt)-Ile-Tyr(tBu)-Thr(tBu)-Lys(Boc)-Lys(Boc)-Glu(OtBu)-Ala-Phe-Asn(Trt)-Val-Glu(OtBu)-Wang. Resin was obtained. The deFmoc reaction and the KURIBEJI reaction were performed to this thing, and 47.4 mg of rough peptide was obtained. :column: ODS column (TSK-gel ODS-120T) which carried out HPLC refining on condition of the following after dissolving the whole quantity in the 20% acetonitrile solution which contains TFA 0.1%

Mobile phase: 39-49% acetonitrile / 0.1% TFA, 30 minutes (linear density gradient)

rate-of-flow: -- a part for 5-ml/-- detection wave length: -- the fraction eluted in 220-nm 22 to 25 minutes was isolated preparatively, freeze-drying was performed after concentration, and 4.4 mg of target peptide was obtained.

[0093] About this refining peptide, the molecular weight was checked by the ESI method using the mass spectrometer. About refining peptide 100pmol, as a result of conducting amino acid sequence analysis from an amino terminal to 10 residue, it was checked that it is in agreement with the amino acid sequence shown in the amino acid numbers 1-10 among the array numbers 4 of an array table.

[0094][Example 5] For the purpose of checking a synthetic situation, and the purpose of adding a reagent to a synthetic machine, composition of the peptide 5 peptide 5 (array number 5 of an array table) was performed by dividing into four steps so that it might indicate below.

1) First by the method indicated in the Example 2. Peptide from a C terminal to 11 residue eye : when Fmoc-Ile-Tyr(tBu)-Thr(tBu)-Lys(Boc)-Lys(Boc)-Glu(OtBu)-Ala-Phe-Asn(Trt)-Val-Glu(OtBu)-Wang resin is compounded, Composition was interrupted after the deFmoc reaction. Resin was extracted in part, the KURIBEJI reaction was performed, and the synthetic product was checked by the method indicated to 1 of Example 1.

[0095] 2) Peptide synthesis is succeedingly performed using the remaining resin of the above 1, Peptide from a C terminal to 31 residue eye: Fmoc-Gly-Leu-Val-His(Trt)-Val-Ala-Asn(Trt)-Asn(Trt)-Asn(Trt)-Tyr(tBu)-Asp(OtBu)-Pro-Ser(tBu)-Gly-Lys(Boc)-Tyr(tBu)-Glu(OtBu)-Gly-Gly-Asn(Trt)-Ile-Tyr(tBu)-Thr(tBu)-Lys(Boc)-Lys(Boc)-Glu(OtBu)-Ala-Phe-Asn(Trt)-Val-Glu(OtBu)-Wang. When resin was compounded, composition was interrupted after the deFmoc reaction. Resin was extracted in part, the KURIBEJI reaction was performed, and the synthetic product was checked by the method indicated to 1 of Example 1.

[0096] 3) Peptide synthesis is succeedingly performed using the remaining resin of the above 2,

Peptide from a C terminal to 54 residue eye: Fmoc-Ala-Ser(tBu)-Lys(Boc)-Asn(Trt)-Phe-His(Trt)-Leu-Gln(Trt)-Lys(Boc)-Asn(Trt)-Lys(Boc)-Leu-Thr(tBu)-Ser(tBu)-Gly-Lys(Boc)-Ile-Ala-Ser(tBu)-Cys(Trt)-Leu-Asn(Trt)-Tyr(tBu)-Gly-Leu-Val-His(Trt)-Val-Ala-Asn(Trt)-Asn(Trt)-Asn(Trt)-Tyr(tBu)-Asp(OtBu)-Pro-Ser(tBu)-Gly-Lys(Boc)-Tyr(tBu)-Glu(OtBu)-Gly-Gly-Asn(Trt)-Ile-Tyr(tBu)-Thr(tBu)-. When Lys(Boc)-Lys(Boc)-Glu(OtBu)-Ala-Phe-Asn(Trt)-Val-Glu(OtBu)-Wang resin was compounded, composition was interrupted after the deFmoc reaction. Resin was extracted in part, the KURIBEJI reaction was performed, and the synthetic product was checked by the method indicated to 1 of Example 1.

[0097]4) Peptide synthesis was succeedingly performed using the remaining resin of the above 3, and peptide from a C terminal to 95 residue eye was compounded. However, Fmoc-Ser(tBu) of a C terminal to 70 residue eye, Fmoc-Lys of 71 residue eye (Boc), The reaction which combines Fmoc-Trp(Boc) of 72 residue eye, and Fmoc-Ser(tBu) of 73 residue eye, Since it was predicted that reaction efficiency is not good, the reaction by a deprotection solution was repeated further 3 times, and after making it the Fmoc basis combined with peptide fully separate, the reaction time which combines amino acid was extended in 120 minutes, and was performed.

[0098]By the above method, it is Fmoc-Gln(Trt)-Phe-Ala-Lys(Boc)-Leu-Thr(tBu)-Gly-Phe-Thr(tBu)-Leu-Met-Gly-Gly-Ile-Ile-Ala-Ala-Tyr(tBu)-Gln(Trt)-Asn(Trt)-Pro-Ala-Ser(tBu)-Trp(Boc)-Lys(Boc)-Ser(tBu)-Met-Lys(Boc)-Val-Thr(tBu)-Val-Ala-Phe-Asn(Trt)-Gln(Trt)-Phe-Gly-Pro-Asp(OtBu)-Ile-Phe-Ala-Ser(tBu)-Lys(Boc)-Asn(Trt)-Phe-His(Trt)-Leu-Gln(Trt)-Lys(Boc)-Asn(Trt)-Lys(Boc)-Leu-Thr(tBu)-Ser(tBu)-Gly-Lys(Boc)-Ile-Ala-Ser(tBu)-Cys(Trt)-Leu-Asn(Trt)-Tyr(tBu)-Gly-Leu-Val-His(Trt)-Val-Ala-Asn(Trt)-Asn(Trt)-Asn(Trt)-Tyr(tBu)-Asp(OtBu)-Pro-Ser(tBu)-Gly-Lys(Boc)-Tyr(tBu)-Glu(OtBu)-Gly-Gly-Asn(Trt)-Ile-Tyr(tBu)-Thr(tBu)-Lys(Boc)-Lys(Boc)-Glu(OtBu)-Ala-Phe-Asn(Trt)-Val-Glu(OtBu)-Wang resin was obtained. The deFmoc reaction and the KURIBEJI reaction were performed to this thing, and 134 mg of rough peptide was obtained. :column:ODS column (TSK-gel ODS-120T) which carried out HPLC refining on condition of the following after dissolving the whole quantity in the 20% acetonitrile solution which contains TFA 0.1%

Mobile phase: 38-50% acetonitrile / 0.1% TFA, 30 minutes (linear density gradient)

rate-of-flow: — a part for 5-ml/— detection wave length: — the fraction eluted in 230-nm 20 to 23 minutes was isolated preparatively, freeze-drying was performed after concentration, and 14 mg of target peptide was obtained.

[0099]About this refining peptide, the molecular weight was checked by the ESI method using the mass spectrometer. About refining peptide 100pmol, as a result of conducting amino acid sequence analysis from an amino terminal to 10 residue, it was checked that it is in agreement with the amino acid sequence shown in the amino acid numbers 1-10 among the array numbers 5 of an array table.

[0100][Example 6] For the purpose of checking a synthetic situation, and the purpose of adding a reagent to a synthetic machine, composition of the peptide 6 peptide 6 (array number 6 of an array table) was performed by dividing into five steps so that it might indicate below.

1) First by the method indicated in the Example 2. Peptide from a C terminal to 21 residue eye: Fmoc-Asp(OtBu)-Pro-Ser(tBu)-Gly-Lys(Boc)-Tyr(tBu)-Glu(OtBu)-Gly-Gly-Asn(Trt)-Ile-Tyr(tBu)-Thr(tBu)-Lys(When Boc-Lys(Boc)-Glu(OtBu)-Ala-Phe-Asn(Trt)-Val-Glu(OtBu)-Wang resin was compounded, composition was interrupted after the deFmoc reaction. Resin was extracted in part, the KURIBEJI reaction was performed, and the synthetic product was checked by the method indicated to 1 of Example 1.

[0101]2) Peptide synthesis is succeedingly performed using the remaining resin of the above 1, Peptide from a C terminal to 54 residue eye: Fmoc-Ala-Ser(tBu)-Lys(Boc)-Asn(Trt)-Phe-His(Trt)-Leu-Gln(Trt)-Lys(Boc)-Asn(Trt)-Lys(Boc)-Leu-Thr(tBu)-Ser(tBu)-Gly-Lys(Boc)-Ile-Ala-Ser(tBu)-Cys(Trt)-Leu-Asn(Trt)-Tyr(tBu)-Gly-Leu-Val-His(Trt)-Val-Ala-Asn(Trt)-Asn(Trt)-Asn(Trt)-Tyr(tBu)-Asp(OtBu)-Pro-Ser(tBu)-Gly-Lys(Boc)-Tyr(tBu)-Glu(OtBu)-Gly-Gly-Asn(Trt)-Ile-Tyr(tBu)-Thr(tBu)-. When Lys(Boc)-Lys(Boc)-Glu(OtBu)-Ala-Phe-Asn(Trt)-Val-Glu(OtBu)-Wang resin was compounded, composition was interrupted after the deFmoc reaction. Resin was extracted in part, the KURIBEJI reaction was performed, and the synthetic product was checked by the method indicated to 1 of Example 1.

[0102]3) Peptide synthesis is succeeding performed using the remaining resin of the above 2, Peptide from a C terminal to 74 residue eye: Fmoc-Ala-Ser(tBu)-Trp(Boc)-Lys(Boc)-Ser(tBu)-Met-Lys(Boc)-Val-Thr(tBu)-Val-Ala-Phe-Asn(Trt)-Gln(Trt)-Phe-Gly-Pro-Asp(OtBu)-Ile-Phe-Ala-Ser(tBu)-Lys(Boc)-Asn(Trt)-Phe-His(Trt)-Leu-Gln(Trt)-Lys(Boc)-Asn(Trt)-Lys(Boc)-Leu-Thr(tBu)-Ser(tBu)-Gly-Lys(Boc)-Ile-Ala-Ser(tBu)-Cys(Trt)-Leu-Asn(Trt)-Tyr(tBu)-Gly-Leu-Val-His(Trt)-Val-Ala-Asn(Trt)-Asn(Trt)-Asn(Trt)-Tyr(tBu)-Asp(OtBu)-Pro-Ser(tBu)-Gly-Lys(Boc)-Tyr(tBu)-Glu(OtBu)-Gly-Gly-Asn(Trt)-Ile-. When Tyr(tBu)-Thr(tBu)-Lys(Boc)-Lys(Boc)-Glu(OtBu)-Ala-Phe-Asn(Trt)-Val-Glu(OtBu)-Wang resin was compounded, the composition after a deFmoc reaction was interrupted. However, Fmoc-Ser(tBu) of a C terminal to 70 residue eye, Fmoc-Lys of 71 residue eye (Boc), The reaction which combines Fmoc-Trp(Boc) of 72 residue eye, and Fmoc-Ser(tBu) of 73 residue eye, Since it was predicted that reaction efficiency is not good, the reaction by a deprotection solution was repeated further 3 times, and after making it the Fmoc basis combined with peptide separate thoroughly, the reaction time which combines amino acid was extended in 120 minutes, and was performed. Resin was extracted in part, the KURIBEJI reaction was performed, and the synthetic product was checked by the method indicated to 1 of Example 1.

[0103]4) Peptide synthesis is succeeding performed using the remaining resin of the above 3, Peptide from a C terminal to 94 residue eye: Fmoc-Phe-Ala-Lys(Boc)-Leu-Thr(tBu)-Gly-Phe-Thr(tBu)-Leu-Met-Gly-Gly-Ile-Ile-Ala-Ala-Tyr(tBu)-Gln(Trt)-Asn(Trt)-Pro-Ala-Ser(tBu)-Trp(Boc)-Lys(Boc)-Ser(tBu)-Met-Lys(Boc)-Val-Thr(tBu)-Val-Ala-Phe-Asn(Trt)-Gln(Trt)-Phe-Gly-Pro-Asp(OtBu)-Ile-Phe-Ala-Ser(tBu)-Lys(Boc)-Asn(Trt)-Phe-His(Trt)-Leu-Gln(Trt)-Lys(Boc)-Asn(Trt)-Lys(Boc)-Leu-Thr(tBu)-Ser(tBu)-Gly-Lys(Boc)-Ile-Ala-Ser(tBu)-Cys(Trt)-Leu-Asn(Trt)-Tyr(tBu)-Gly-Leu-Val-His(Trt)-Val-Ala-Asn(Trt)-Asn(Trt)-Asn(Trt)-Tyr(tBu)-Asp(OtBu)-Pro-Ser(tBu)-Gly-Lys(Boc)-Tyr(tBu)-Glu(OtBu)-Gly-Gly-Asn(Trt)-Ile-Tyr(tBu)-Thr(tBu)-Lys(Boc)-Lys(Boc)-Glu(OtBu)-Ala-Phe-Asn(Trt)-Val-Glu(OtBu)-Wang. When resin was compounded, composition was interrupted after the deFmoc reaction. Resin was extracted in part, the KURIBEJI reaction was performed, and the synthetic product was checked by the method indicated to 1 of Example 1.

5) Peptide synthesis was succeeding performed using the remaining resin of the above 4, and peptide from a C terminal to 99 residue eye was compounded.

[0104]To the above method. More, it is Fmoc-Gly-Asp(OtBu)-Pro-Arg-Gln(Trt)-Phe-Ala-Lys(Boc)-Leu-Thr(tBu)-Gly-Phe-Thr(tBu)-Leu-Met-Gly-Gly-Ile-Ile-Ala-Ala-Tyr(t. Bu)-Gln(Trt)-Asn(Trt)-Pro-Ala-Ser(tBu)-Trp(Boc)-Lys(Boc)-Ser(tBu)-Met-Lys(Boc)-Val-Thr(tBu)-Val-Ala-Phe-Asn(Trt)-Gln(Trt)-Phe-Gly-Pro-Asp(OtBu)-Ile-Phe-Ala-Ser(tBu)-Lys(Boc)-Asn(Trt)-Phe-His(Trt)-Leu-Gln(Trt)-Lys(Boc)-Asn(Trt)-Lys(Boc)-Leu-Thr(tBu)-Ser(tBu)-Gly-Lys(Boc)-Ile-Ala-Ser(tBu)-Cys(Trt)-Leu-Asn(Trt)-Tyr(tBu)-Gly-Leu-Val-His(Trt)-Val-Ala-Asn(Trt)-Asn(Trt)-Asn(Trt)-Tyr(tBu)-Asp(OtBu)-Pro-Ser(tBu)-Gly-Lys(Boc)-Tyr(tBu)-Glu(OtBu)-Gly-Gly-Asn(Trt)-Ile-Tyr(tBu)-Thr(tBu)-Lys(Boc)-Lys(Boc)-Glu(OtBu)-Ala-Phe-Asn(Trt)-Val-Glu(OtBu)-Wang. Resin was obtained. The deFmoc reaction and the KURIBEJI reaction were performed to this thing, and 706 mg of rough peptide was obtained. among these, :column:C22 column (docosyl- B) which carried out HPLC refining on condition of the following after dissolving 43 mg in the 20% acetonitrile solution which contains TFA 0.1%

Mobile phase: 32-36% acetonitrile / 0.1% TFA, 30 minutes (linear density gradient)

rate-of-flow: -- a part for 1.5-ml/-- detection wave length: -- the fraction eluted in 230-nm 23 to 25 minutes was isolated preparatively, after concentration, freeze-drying was performed and 3.2 mg of target peptide was obtained.

[0105]About this refining peptide, the molecular weight was checked by the ESI method using the mass spectrometer. About refining peptide 100pmol, as a result of conducting amino acid sequence analysis from an amino terminal to 10 residue, it was checked that it is in agreement with the amino acid sequence shown in the amino acid numbers 1-10 among the array numbers 6 of an array table.

[0106][Example 7] The peptide 7 peptide 7 (array number 7 of an array table) was compounded according to the protocol of the device using the multi-peptide synthesizer (a symphony, protein technology company make) by the Fmoc solid-phase-synthesis method.

[0107]Namely, an equivalent for 25micromol of the Fmoc-Lys(Boc)-Wang resin (0.50 mmol/g) in which the amino acid (Lys) equivalent to the C terminal residue of the peptide to compound is introduced is set to the reaction vessel of the above-mentioned peptide synthesis device, Added 1.25 ml of deprotection solutions (20% piperidine / dimethylformamide), it was made to react twice for 5 minutes, and the Fmoc basis of the amino acid combined with resin was removed. 1.25 ml of dimethylformamide liquid washes resin 6 times for 30 seconds, In 1.25 ml of 100mM solutions (a solvent is dimethylformamide) of derivative Fmoc-Trp (Boc) of the 2nd amino acid, from the C terminal side. The activator solution for multi-peptide synthesizers. (100mM2-(1H-benzotriazol 1-yl)-1,1,3,3-tetramethyl *****- hexafluorophosphate / 400mM N-methyl morpholine / dimethylformamide) 1.25 ml is added (it is 5 time equivalent weight to joint amino acid), It was made to react at a room temperature for 20 minutes. The Fmoc-Trp(Boc)-Lys (Boc)-Wang resin generated here by 1.25 ml of dimethylformamide After 6 times washing during 30 seconds, A Fmoc basis is removed again, and the Fmoc-Ser (tBu) solution and the activator solution for multi-peptide synthesizers were added, and were made to react after 6 times washing during 30 seconds by 1.25 ml of dimethylformamide. repeating the same operation -- protection peptide resin: -- Fmoc-Gly-Ile-Ile-Ala-Ala-Tyr(tBu)-Gln (Trt) (-Asn(Trt)-Pro-Ala-Ser(tBu)-Trp(Boc)-Lys(Boc)-Wang resin was compounded.)

[0108]: which is as follows [amino acid derivative / which was used for composition of the peptide 7 and the peptide 8 (array number 8 of an array table)], [Fmoc-Ala and] Fmoc-Asn (Trt), Fmoc-Asp (OtBu), Fmoc-Cys (Trt), Fmoc-Gln (Trt), Fmoc-Glu (OtBu), Fmoc-Gly, Fmoc-His (Trt), Fmoc-Ile, Fmoc-Leu, Fmoc-Lys (Boc), Fmoc-Met, Fmoc-Phe, Fmoc-Pro, Fmoc-Ser (tBu), Fmoc-Thr (tBu), Fmoc-Trp (Boc), Fmoc-Tyr (tBu), Fmoc-Val (the inside of () expresses the protective group which protects the reaction group of a residue portion.) ; The product made from a PerkinElmer Japan Applied Bio-systems operation division.

[0109]1.25 ml of deprotection solutions were made to react to the protection peptide resin which might be compounded as mentioned above twice for 5 minutes, and deprotection of the Fmoc basis of an amino terminal was carried out. Next, dichloromethane washed resin 9 times after 6 times washing in 1.25 ml of dimethylformamide, and it was made to dry for 20 minutes by spraying nitrogen gas further. Collect resin and 2 ml of KURIBEJI solutions (ethanedithiol: TFA : water : triisopropyl silane =92.5:2.5:2.5:2.5 (volume ratio)) are added, By making it react at a room temperature for 2 hours, cutting of peptide from resin and removal of the amino acid side chain protective group were performed, and the peptide solution was obtained. This peptide solution was filtered using the PTFE filter, and filtrate was collected to the centrifuging tube. 10 ml of cold ether was added to this filtrate, and peptide was settled. The operation which carries out centrifugality of this, collects precipitate (for 3000 rpm and 10 minutes) after cooling for a while, and are collected if cold ether is added again and it is made to distribute was repeated 4 times, and peptide washing was carried out. The obtained peptide was dried and rough peptide was obtained.

[0110]:column:C22 column which carried out HPLC refining on condition of the following after dissolving 7.0 mg of the obtained rough peptide in the 20% acetonitrile solution which contains TFA 0.1% (docosyl- B)

Mobile phase: 20-25% acetonitrile / 0.1% TFA, 20 minutes (linear density gradient)

rate-of-flow: -- a part for 7-ml/-- detection wave length: -- the fraction eluted in 220-nm 16 to 17 minutes was isolated preparatively, freeze-drying was performed after concentration, and 2.0 mg of target peptide was obtained. About this refining peptide 100pmol, as a result of conducting amino acid sequence analysis, the amino acid sequence shown in the array number 7 of an array table was checked.

[0111][Example 8] By the same operation as peptide 8 Example 7. Protection peptide resin : Fmoc-Asp(OtBu)-Ile-Phe-Ala-Ser(tBu)-Lys(Boc)-Asn(Trt)-Phe-His(Trt)-Leu-Gln(Ttrt)-Lys (Boc)-Asn(Trt)-Wang resin is compounded, Deprotection and a KURIBEJI reaction were performed and rough peptide was obtained.

[0112]:column:ODS column (TSK gel ODS-120T) which carried out HPLC refining on condition of the following after dissolving 3.1 mg of the obtained rough peptide in the 20% acetonitrile solution which contains TFA 0.1%

Mobile phase: 22–27% acetonitrile / 0.1% TFA, 20 minutes (linear density gradient)
rate-of-flow: — a part for 2-ml/— detection wave length: — the fraction eluted in 220-nm 14 to 15.5 minutes was isolated preparatively, freeze-drying was performed after concentration, and 0.9 mg of target peptide was obtained. About this refining peptide 100pmol, as a result of conducting amino acid sequence analysis, the amino acid sequence shown in the array number 8 of an array table was checked.

[0113][example 9] peptide 91 That with which the synthetic peptide 9 (amino acid numbers 1–185 of the array number 10 of an array table) of DNA which encodes a basic unit connected six sorts of T cell epitopes of cedar pollen allergen like A–E–C–D–F–G (henceforth “H6–1”) The array number 31 of an array table, and linker peptide:Thr–Met–Ile–Thr–Asn–Ser–Ser–Ser–Val–Pro–Gly–Asp–Pro–Arg which has the following amino acid sequence (L1, array number 26 of an array table);

Arg–Ala–Asp–Pro–Arg; (L2, array number 27 of an array table) and Arg–Ala–Asp–Leu (L1), –(H6–1)–(L2)–(H6–1)– (L3) (L3, the array number 80 of an array table)

** — it has the structure connected like. In making *Escherichia coli* produce the peptide 9 using gene modification technology, DNA (henceforth “h6–1”) which encodes H6–1 first is prepared so that it may explain in full detail below, Subsequently, by polymerizing the field containing DNA which encodes H6–1, the manifestation plasmid vector for *Escherichia coli* containing DNA which encodes the peptide 9 was built, and the method of transforming *Escherichia coli* by this plasmid vector was used.

[0114]In the design of h6–1, the codon usage [Crantham, R. etal. (1981) *Nucleic Acids Res.* 9, and 43] of *Escherichia coli* is taken into consideration, The restriction enzyme recognition sequence required in order to make h6–1 polymerize behind was added to the five prime end [of a coding region], and three-dash terminal side (BamHI) (BglII), respectively. In consideration of insertion to an expression plasmid, the restriction enzyme SalI recognition sequence was added to the pan of the three-dash terminal side BglII recognition sequence at the three-dash terminal side (array number 30 of an array table).

[0115]first, Following oligonucleotide:5′- gatccgcgtg gtatcatcgc agcataccag aaccgcggcat which is the partial sequence of the sense strand of h6–1, or an antisense strand to which the restriction enzyme recognition sequence was added. cttgg–3′ (F1, array number 32 of an array table);

5′- catagaccaa gatgccgggt tctggtatgc tgcgatgata ccacgcg–3′ (F2, array number 33 of an array table);

5′- tctatgaaag ttaccgttgc ttcaaccag ttcggtccg–3′ (F3, array number 34 of an array table);

5′- tgcgaacgga cgaactggt tgaaagcaac ggtaacttt–3′ (F4, array number 35 of an array table);

5′- ttgcaccta aaaacttcca tctgcagaaa–3′ (F5, array number 36 of an array table);

5′- ggtgttttc tgcgatgga agtttttaga–3′ (F6, array number 37 of an array table);

5′- aacaccaaac tgacctctgg taaaatcgca tottgc–3′ (F7, array number 38 of an array table);

5′- gttcaggcaa gatgcgattt taccagaggt cagttt–3′ (F8, array number 39 of an array table);

5′- ctgaactacg gtctgtttca tgttgcaaac aacaactacg a–3′ (F9, array number 40 of an array table);

5′- gacgggtcgt agttgtttt tgcaacatga accagaccgt a–3′ (F10, array number 41 of an array table);

5′- cccgtctggt aaatacgaag gtgtaacat ctacacacaaa a–3′ (F11, array number 42 of an array table);

5′- cttctttttt ggtgtagatg ttaccacctt cgtatttacc a–3′ (F12, array number 43 of an array table);

5′- aagaagcatt caacgttgaa. cgtgcagatc tgtaag–3′(F13, array number 44 of array table); and 5′-

tgcacttaca gatctgcacg ttcaacgttg aatg–3′ (F14, array number 45 of an array table)

It compounded with the DNA synthesis machine (model 394; made by an applied bio-systems company). These are combination of F1, F2 and F3, F4 and F5, F6 and F7, F8 and F9, F10 and F11, F12 and F13, and F14, It is complementary in the nucleotide sequence by the side of a three-dash terminal from the 7th nucleotide from the five prime end side respectively, and complementary respectively in six nucleotide sequences by the side of a five prime end with the combination of F3, F2, F5, F4 and F7, F6 and F9, F8 and F11, F10 and F13, and F12. In therefore, the combination first of F1, F2 and F3, F4 and F5, F6 and F7, F8 and F9, F10 and F11, F12 and F13, and F14. After carrying out annealing of the complementary strand, respectively and forming double stranded DNA, h6–1 is producible by connecting each fragment using the complementary strand of the 5′-cohesive end produced in 2 each chain DNA.

[0116] Nitrogen was blown, each compound oligonucleotide removed ammonia, after making it dry, it dissolved in sterile distilled water, and extracted the 10–30 microg, and performed electrophoresis by 8% polyacrylamide gel which contains 7M urea, respectively. starting the gel of the band part containing each oligonucleotide — an elution buffer (0.5M ammonium acetate, 10mM magnesium acetate) — in addition, shaking, it was kept warm at 37 °C for 16 hours, and the oligonucleotide was made eluted Hiroshi's cold ethanol is added to this eluate 2.5 times, and the oligonucleotide was settled, and at 10000xg and 4 °C, it centrifuged for 15 minutes and collected. It dissolved in the sterile distilled water of 20 microl after washing by ethanol 70%, and precipitate was made into refining oligonucleotides.

[0117] Next, the five prime end of F1 and the oligonucleotide of F2–F13 excluding F14 was phosphorylated, respectively (following "F2p" it is called – "F13p"). 5–10 micro of oligonucleotide (F2–F13) 10 times concentration which was as indicating a reaction mixture presentation below (T4 polynucleotide-kinase (it attaches to TAKARA SHUZO [CO., LTD.] CO., LTD. make) 5 microl 10mM.) Buffer solution for phosphotransfer reactions Adenosine triphosphate (henceforth "ATP") 5 microl T4 Polynucleotide kinase (made by TAKARA SHUZO [CO., LTD.] CO., LTD.) It was referred to as 50 microl with ten-unit sterile distilled water.

Temperature conditions: It was kept warm for 5 minutes at 70 °C after 1-hour incubation at 37 °C, the enzyme was deactivated, and phosphorylation was stopped.

[0118] To each reaction mixture after ending reaction, 3M sodium acetate solution was added 1/10, cold ethanol was centrifuged for 15 minutes at 10000xg and 4 °C after 2.5 times to it, and F2 p–F 13p was collected as precipitate to it (this operation is hereafter called "ethanol precipitate"). It dissolved in the sterile distilled water of 5 microl after washing by ethanol 70%.

[0119] Subsequently, F2 p–F 13p, and F1 and F14 were summarized in one tube, and annealing was performed. oligonucleotide fragment which was as indicating a reaction mixture presentation below (F1, F2 p–F 13p, F14) each 2.5 –5 microl 1M — tris-chloride buffer solution (pH 7.5) 10 microl 1M magnesium chloride solution The whole quantity was set to 100 microl with 1 microl distillation sterilized water.

Temperature conditions: Thermal cycler (model 9600; made by Perkin-Elmer) was used, and it cooled to the heating back for 5 minutes at 95 °C, and cooled from 95 °C to 25 °C in 90 minutes.

[0120] 10mM which performs ethanol precipitate, collects precipitate after ending reaction, and contains 1mM ethylenediaminetetraacetic acid (henceforth "EDTA") It dissolved in 10 micro of tris-chloride buffer solution (it is called below pH8.0: "TE buffer solution") 1. It was kept warm in the description attached to the kit for 16 hours, and 3 micro of oligonucleotide mixed liquor 1 which carried out annealing was made to connect with it at 16 °C in accordance with the method of a statement with a DNA ligation kit (made by TAKARA SHUZO [CO., LTD.] CO., LTD.). After the end of ligation, ethanol precipitate was performed, precipitate was collected, and it dissolved in the sterile distilled water of 6 microl after washing by ethanol 70%.

[0121] On the other hand, after digesting cloning vector pUC18 (made by TAKARA SHUZO [CO., LTD.] CO., LTD.) one by one with the restriction enzymes BamHI and SalI, ethanol precipitate was performed, precipitate was collected and it dissolved in TE buffer solution after washing by ethanol 70%. Low melting point agarose gel (made by FMC bioproducts company) electrophoresis was performed 0.8% about this thing. DNA in gel will be visualized by the well-known method to a person skilled in the art after electrophoresis, the gel of the band part equivalent to about 2.7 kbp(s) will be started, and the final concentration of agarose will be 0.5% or less — as — TE buffer solution (pH 8.0) of isochore — in addition, it was kept warm for 5 minutes at 65 °C, and the piece of gel was melted. This with the phenol solution (what added the eight quinolinol to saturated phenol so that it might become 0.1% with final concentration) of isochore 2 times, Phenol chloroform fluid (what mixed a phenol solution, chloroform, and isoamyl alcohol to 50 to 49 to 1) extracted once with chloroform fluid (what mixed isoamyl alcohol with chloroform to 49 to 1). Furthermore ethanol precipitate was performed, precipitate was collected and it dissolved in sterile-distilled-water 10 mul after washing by ethanol 70%. This pUC18 solution that carried out BamHI–SalI digestion The fragment which connected above-mentioned F1 – F14 was

connected with 1microl using 2microl, in addition a DNA ligation kit.

[0122]In addition to competent Escherichia coli JM[109 share (made by TAKARA SHUZO / CO., LTD. / CO., LTD.) / 100micro] I prepared by the Hanahan method [Hanahan, D.(1983) J.Mol. Biol.166, and 557 -580 reference], this ligation reaction mixture was ice-cooled for 30 minutes. It ice-cooled promptly for 3 minutes after keeping it warm for 45 seconds at 42 **. The SOC culture medium of 900microl was added to this, and shaking culture was carried out at 37 ** for 1 hour. This culture medium was applied on L-broth agar-medium plate which contains ampicillin 50 microg/ml, and was opened, and it cultivated at 37 ** overnight. :L culture medium which is as indicating below the presentation of the culture medium used here Bacto trypton (made by Difco) 10g bacto yeast extract (made by Difco) 5g sodium chloride 5 g of whole quantity was 1 l. with ion exchange water.

[0123]

L-broth agar medium 10 g of bacto trypton Bactoyeast extract 5g sodium chloride 5 g Bacto agger (made by Difco) The whole quantity was 1 l. with 15g ion exchange water.

[0124]

2xTY culture medium Bacto trypton 16g bactoyeast extract 10g sodium chloride 5 g of whole quantity was 1 l. with ion exchange water.

[0125]

SOC culture medium Bacto trypton 20 g. Bactoyeast extract 5g 5M sodium chloride solution 2ml 2M potassium chloride solution 1.25ml 1M magnesium chloride solution 10ml 1M magnesium sulfate solution With ion exchange water, 10 ml of 10-ml2M grape sugar solutions the whole quantity. It could be 1 l.

[0126]It isolated, the ampicillin tolerance colony which appeared in the above-mentioned culture condition was inoculated into L-broth culture medium, and shaking culture was carried out at 37 ** overnight. From 3 ml of culture medium, the QIAGEN plasmid mini kit (made by QIAGEN K.K.) is used for the description attached to the kit in accordance with the method of a statement, Cloning vector pUC18 (henceforth "pUC (h6-1)") containing h6-1 was extracted (in addition (drawing 1), the above-mentioned kit was used for all the plasmid extraction in the example indicated below). After ethanol precipitate, the plasmid extracted with the kit carried out ethanol washing 70%, and dissolved in sterile-distilled-water 50mul.

[0127]Thus, the nucleotide sequence of DNA inserted in obtained pUC (h6-1), As a result of investigating using a DNA sequencer (a 310 genetic analyzer, applied bio-systems company make), h6-1 by which cloning was carried out, Although c of the nucleotide number 247 of the nucleotide sequence (array number 30 of an array table) designed at the beginning was replaced by t, Since change was not given to the codon of amino acid Asn (amino acid number 79 of the array number 31 of an array table) by which a code is carried out to the part, it was used for the following examples as it is. The amino acid sequence by which a code is carried out to the nucleotide sequence and this arrangement of h6-1 after substitution was shown in the array number 24 (nucleotide numbers 11-253) and the array number 25 (amino acid numbers 4-84) of the array table, respectively.

[0128]2) The outline of construction of a Japan cedar allergen epitope 6 connection peptide H6-1 polymerization protein expression plasmid is shown in construction drawing 1 of peptide 9 expression plasmid. pUC (h6-1) obtained by the above 1 was digested with the restriction enzymes BamHI and SalI, and the small fragment (an equivalent for about 270 bp(s)) was refined 2.5% by the electrophoresis using low melting point agarose gel (made by a FMC bioproducts company). Apart from this, pUC (h6-1) was digested by BglII and SalI, and the large fragment (an equivalent for about 2.9 kbp(s)) was refined 1% by low melting point agarose gel (made by FMC bioproducts company) electrophoresis. Next, plasmid pUC(h6-1) ₂ was obtained by using a DNA ligation kit for the BglII-SalI digestive large fragment of pUC (h6-1), and connecting the BamHI-SalI digestive smallness fragment of pUC (h6-1) with it. Since the cohesive end produced by BamHI digestion or BglII digestion is the same, a BamHI digestive fragment and a BglII digestive fragment can be connected, but the nucleotide sequence after the connection is not re-cut by BamHI or BglII.

[0129]3) 21 shares (even 109 shares of JM are good) of manifestation Escherichia coli YA in Escherichia coli were applied on L-broth agar-medium plate, and was opened, and it cultivated at 37 ** overnight. Inoculation of the formed single colony was carried out to the 3-l. Erlenmeyer flask containing a 250-ml SOB culture medium, and shaking culture was carried out at 18 ** and 200 rpm. 45 hours afterward, the flask was attached in ice water and it cooled for 10 minutes in the place where the absorbance (OD_{660nm}) at 660 nm of culture medium amounted to 1.19.

1200xg and the biomasses which centrifuged for 15 minutes and precipitated were collected for this culture medium at 4 **. This biomass was suspended to 80 ml of ice-cooling transformation buffer solution (henceforth "TB"), and 1200xg and the biomasses which centrifuged for 15 minutes and precipitated were collected at 4 ** after ice-cooling for 10 minutes. a biomass is re-suspended to 20 ml of ice-cooling TB -- dimethyl sulfoxide (henceforth "DMSO") -- 1.5 ml (7% of final concentration) -- in addition, it ice-cooled for 10 more minutes. 0.5 ml of this thing was poured distributively in each tube in the dry ice ethanol bath, and it froze promptly, and kept at -80 ** as a competent Escherichia coli sample. The presentation of the buffer solution and the culture medium which were used here, :SOB culture-medium: which was as indicating below -- bacto trypton 20g bactoyeast extract 5g 5M sodium chloride solution 2ml 2M potassium chloride solution 1.25ml 1M magnesium chloride solution . 10ml 1M magnesium sulfate solution 10 ml of whole quantity was 1 l. with ion exchange water.

[0130]

Transformation buffer solution (TB): PIPES (made by Nacalai Tesque, Inc.) A 3.0g calcium chloride and 2 hydrate 2.2g potassium chloride A 18.6g manganese chloride and 4 hydrate 10.9 g of whole quantity shall be 1 l. with distilled water, 5N potassium hydroxide solution adjusted pH to 6.7-6.8.

[0131]Plasmid pUC(h6-1) ₂ built by the above 2, Since the lacZ gene under lac promotor rule of pUC18 and DNA which encodes the peptide 9 are connected on the same reading frame, the manifestation of the peptide 9 is possible by introducing these plasmids into a host (drawing 2).

[0132]pUC(h6-1) ₂ of 60ng was ice-cooled for 3 minutes after keeping it warm for 45 seconds at 42 **, immediately after ice-cooling for 30 minutes in addition to competent Escherichia coli 100mul prepared as mentioned above. The SOC culture medium of 900microl was added to this, and shaking culture was carried out at 37 ** for 1 hour. This culture medium was applied on L-broth agar-medium plate containing 50 microg/ml ampicillin, and was opened, and it cultivated at 37 ** overnight. 2 ml of 2xTY culture media which include the ampicillin tolerance colony which appeared for 50 microg/ml ampicillin performed shaking culture at 37 **. 8 hours afterward, it moved to 100 ml of 2xTY culture media containing 100 microg/ml ampicillin, and shaking culture was performed at 37 ** for 14 hours so that last OD_{660nm} might become 0.01 in a part of the culture medium in the place where OD_{660nm} of culture medium amounted to 1-3. Polymerization protein formed the inclusion body within the biomass of 21 shares of Escherichia coli YA at the time of the end of culture.

[0133]90-ml culture medium was taken after the end of culture, and the biomasses which perform 6000xg, 4 **, and centrifugal separation for 20 minutes, and precipitate were collected. After the biomass was suspended to 4.5 ml of TES buffer solution, and crushing a biomass using an ultrasonic homogenizer (SONIFAIA 250, made in Branson), it centrifuged for 20 minutes at 13000xg and 4 **, and the inclusion body was obtained as precipitate. Since the biomass fragment was still contained in this precipitate fraction so much, after being suspended to 4.5 ml of phosphate buffer solutions which contain the triton X-100 1% and shaking for 30 minutes at a room temperature, it centrifuged for 20 minutes at 25000xg and 4 **, and precipitate was collected. After repeating this operation 4 times, 9 ml of distilled water washed the precipitate fraction twice. 4.5-ml 6M guanidine salt acid solution (pH 7.6) was added to the moiety of the precipitate fraction, it shook at 37 ** for 1 hour, and precipitate was solubilized, and after centrifugal separation and supernatant liquid were collected for 20 minutes at 25000xg and 4 **, and it was considered as the rough refining liquid of the peptide 9. :TES buffer solution which was as indicating below the presentation of the buffer solution used here (pH 7.6) : 1M Tris-

chloride buffer solution (pH 7.6) 10ml 5M sodium chloride solution 30ml 0.5M EDTA solution 20 ml of whole quantity shall be 1 l. with distilled water.

[0134]

Phosphate buffer solution (X-1% triton 100 content) : Sodium chloride 8g potassium chloride 0.2g disodium hydrogenphosphate and 12 hydrate 2.9g potassium dihydrogen phosphate 0.2 g Triton X-100 (made by a sigma company) The whole quantity was 1 l. with 10g distilled water.

[0135]

6M guanidine-salt acid solution (pH 7.6): Guanidinium hydrochloride 57.32g 1M Tris-chloride buffer solution (pH 7.6) 5ml 1M dithiothreitol (henceforth "DTT") solution 1 ml of whole quantity was 100 ml with distilled water.

[0136]:column which performed HPLC refining on condition of the following about rough refining liquid 75mul of the above-mentioned peptide 9: ODS column (TSK-gel ODS-120T, phi7.8mmx300mm, TOSOH [CORP.] CORP. make)

Mobile phase: 20% acetonitrile / 0.1% TFA (0 to 5 minutes), 20-70% acetonitrile (5 to 35 minutes) / 0.1% TFA (linear density gradient)

rate-of-flow: — a part for 2-ml/— detection wave length: — about the substance obtained by isolating a neighboring peak preparatively for 220-nm elution time 24.3 minutes, as a result of conducting amino acid sequence analysis from an amino terminal to 25 residue, it was in agreement with the amino acid sequence shown in the amino acid numbers 1-25 of the array number 10 of an array table. The methionine residue of the amino terminal of the amino acid sequence by which a code is carried out to h6-1 was lost in the process to refining. About the obtained substance, as a result of measuring a molecular weight by the ESI method, it was in agreement with the molecular weight predicted from the amino acid composition of the peptide 9. 4.1 mg per 100 ml of culture medium of peptide 9 was obtained as mentioned above.

[0137][example 10] peptide 101 That with which the synthetic peptide 10 (amino acid numbers 1-209 of the array number 12 of an array table) of DNA which encodes a basic unit connected seven sorts of T cell epitopes of cedar pollen allergen like A-E-C-D-F-G-B (henceforth "H7-1") The array number 29 of an array table, and the linker peptide L1, L2 and L3 which were indicated in Example 9 (L1), -(H7-1)-(L2)-(H7-1)- (L3)

** — it has the structure connected like. In making Escherichia coli produce the peptide 10 using gene modification technology, DNA (henceforth "h7-1") which encodes H7-1 first is prepared so that it may explain in full detail below, Subsequently, by polymerizing the field containing DNA which encodes h7-1, the manifestation plasmid vector for Escherichia coli containing DNA which encodes the peptide 10 was built, and the method of transforming Escherichia coli by this plasmid vector was used.

[0138]In the design of h7-1, the codon usage [Crantham, R. etal. (1981) Nucleic Acids Res. 9, and 43] of Escherichia coli is taken into consideration, The restriction enzyme recognition sequence required in order to make h7-1 polymerize behind was added to the five prime end [of a coding region], and three-dash terminal side (BamHI) (BglII), respectively. In consideration of insertion to an expression plasmid, the restriction enzyme SalI recognition sequence was added to the pan of the three-dash terminal side BglII recognition sequence at the three-dash terminal side (array number 28 of an array table).

[0139]First, as a partial sequence of the sense strand of h7-1 to which the restriction enzyme recognition sequence was added, or an antisense strand, It adds to F1 compounded in Example 9 - F12, and is further following oligonucleotide:5'- aagaagcatt caacgttgaacagttcgcta aactg-3' (F15, array number 46 of an array table);

5'- accggtcagt tttagcgaact gttcaacgtt gaatg-3' (F16, array number 47 of an array table);

5'- accggtttca ccctgatggg. tcgtgcagat ctgtaag-3'(F17, array number 48 of array table); and 5'- togacttaca gatctgcacg acccatcagg gtgaa-3' (F18, array number 49 of an array table)

It compounded with the DNA synthesis machine and refined by the method indicated to 1 of Example 9. The five prime end was phosphorylated by the method which indicated F15, F16, and F17 to 1 of Example 9 (henceforth "F16p" and "F17p" "F15p").

[0140]Next, annealing of the 2.5microeach l of F2 p-F 12p and F15 p-F 17p which phosphorylated oligonucleotide F1, F18, and a five prime end was carried out by a presentation

and temperature conditions of Example 9 given in one, and it dissolved in TE buffer solution 10μl after ethanol precipitate. The 3μl was connected using the DNA ligation kit, and it dissolved in TE buffer solution 5μl after ethanol precipitate. In order to perform PCR by using this connected fragment as a mold on the other hand, it is following oligonucleotide: 5'-ggatccgcgt ggtatcatcg ca-3' () as a primer. [PRv and] Array number 50; and 5'- aggtcgactt acagatctgc ac-3' (PFW, array number 51 of an array table) of an array table

It compounded.

[0141] It ranks second, On the reaction mixture presentation and conditions which are indicated below, PCR. carried-out : — template DNA: — synthetic fragment after connection . 2μl 10 time concentration Ex Taq PCR buffer solution . (Ex Taq It attaches to polymerase (made by TAKARA SHUZO [CO., LTD.] CO., LTD.)) 10μl dNTP mixed liquor (Ex Taq it attaches to polymerase) — 8μl 20 pmol/μl primer PRv 2.5μl 20 pmol/μl — primer PFW. 2.5μl Ex Taq The whole quantity was set to 100μl with 2.5 units of polymerase (made by TAKARA SHUZO [CO., LTD.] CO., LTD.) sterile distilled water (dNTP mixed liquor expresses the equimolar mixture of dATP, dCTP, dGTP, and dTTP.). It is the same about the following statements.

Temperature conditions : After heat-treating for 3 minutes at 94 **, at 55 ** for 30 seconds at 94 ** 30 seconds, After repeating the cycle of 1 minute 30 times at 72 **, it was kept warm for 10 minutes at 72 ** (reaction temperature regulation of all the PCR in the example indicated below used thermal cycler model 9600; Perkin-Elmer make).

[0142] After ending reaction, 50μl and isopropanol are 150μl Added for 10M ammonium acetate solution, and DNA was settled, and after centrifuging and collecting (this operation is called "isopropanol precipitate" below), it washed by ethanol 70% and dissolved in sterile-distilled-water 20μl. After digesting this thing (2μl) with the restriction enzyme BamHI and digesting by SalI further, ethanol precipitate was performed, precipitate was collected and it dissolved in TE buffer solution of 4μl after washing by ethanol 70%. This DNA fragment solution (2μl) that carried out BamHI-SalI digestion, and the pUC18 vector solution (1μl) which were prepared by 1 of Example 9 and which carried out BamHI-SalI digestion were connected using the DNA ligation kit. 109 shares of competent Escherichia coli JM were transformed by the method indicated to 1 of Example 9 by this ligation reaction mixture, and it cultivated at 37 **. It isolated, the ampicillin tolerance colony which appeared was inoculated into L-broth culture medium, and shaking culture was carried out at 37 ** overnight. The plasmid pUC (h7-1) containing h7-1 was extracted from 3 ml of this culture medium (drawing 1). Ethanol precipitate of the extracted plasmid was carried out, and it dissolved in sterile-distilled-water 50μl after 70% ethanol washing. As a result of investigating the nucleotide sequence of h7-1 inserted in pUC (h7-1), it was checked that it is a nucleotide sequence (array number 28 of an array table) which encodes H7-1 (array number 29 of an array table).

[0143] 2) The outline of construction of a Japan cedar allergen epitope 7 connection peptide H7-1 polymerization protein expression plasmid is shown in construction drawing 1 of the expression plasmid which encodes the peptide 10. pUC (h7-1) prepared by the above 1 was digested with the restriction enzymes BamHI and SalI, and the small fragment (about 300 bp(s)) was refined by the electrophoresis using low melting point agarose gel 2.5%. On the other hand apart from this, pUC (h7-1) was digested with the restriction enzymes BglII and SalI, and the large fragment (about 3.0 kbp(s)) was refined by low melting point agarose gel electrophoresis 1.0%. Next, plasmid pUC(h7-1)₂ in which DNA (array number 11 of an array table) which encodes the peptide 10 was inserted was obtained by connecting these fragments using a DNA ligation kit.

[0144] 3) 21 shares (even 109 shares of JM are possible) of competent Escherichia coli YA were transformed by pUC(h7-1)₂ of 10ng like 3 of the manifestation example 9 in Escherichia coli.

Shaking culture of this transformant was carried out at 37 ** in 5 ml of L-broth culture media containing 50 microg/ml ampicillin. 9 hours afterward, inoculation was carried out to the 1-l. 2xTY culture medium containing 100 microg/ml ampicillin, and shaking culture was performed at 37 ** for 14 hours so that last OD_{660nm} might become 0.01 in a part of this culture medium in

the place where OD_{660nm} of culture medium amounted to three or more. Polymerization protein formed the inclusion body within the biomass of 21 shares of YA(s) at the time of the end of culture. 90 ml of this culture medium was taken, and 6000xg and the biomasses which it centrifuges for 20 minutes and precipitate at 4 °C were collected. It was suspended to 9 ml of TES buffer solution, the biomass was centrifuged for 20 minutes at 13000xg and 4 °C after crushing with the ultrasonic homogenizer, and the inclusion body was obtained as precipitate. 9 ml of triton content phosphate buffer solutions washed precipitate 4 times 1%. 4.5 ml of 2M urea buffer solution (2M urea, 50mM tris-chloride (pH 7.6), 10mM DTT) washes the amount of halves of precipitate twice with 4.5 ml of distilled water, It solubilized with 4.5 ml of 6M guanidine salt acid solutions, and supernatant liquid was used as the rough refining liquid of the peptide 10 after the centrifugal separation for 20 minutes at 25000xg and 4 °C. :column:C18 column which performed HPLC refining on condition of the following about 1 ml of this rough refining liquid (capsule pack C18 SG300, phi10mmx250mm, Shiseido Make)

Mobile phase: 37–42% acetonitrile / 0.1%TFA, 30 minutes (linear density gradient)

rate-of-flow: — a part for 3-ml/— detection wave length: — about the substance produced by isolating a neighboring peak preparatively for 220-nm elution time 13.9 minutes, as a result of conducting amino acid sequence analysis from an amino terminal to 38 residue, it was in agreement with the amino acid sequence shown in the amino acid numbers 1–38 of the array number 12 of an array table. The methionine residue of the amino terminal of the amino acid sequence by which a code is carried out to h7-1 was lost in the process to refining. About the obtained substance, as a result of measuring a molecular weight by the ESI method, it was in agreement with the molecular weight predicted from the amino acid composition of the peptide 10. 27 mg per l. of culture medium of peptide 10 was obtained as mentioned above.

[0145][Example 11] The synthetic peptide 12 (peptide which consists of an amino acid sequence shown in the amino acid numbers 1–95 of the array number 15 of an array table) of DNA which encodes the peptide 11 and 121 basic unit, Having the structure which connected seven sorts of T cell epitopes of cedar pollen allergen like A'-E-C'-D-F-G-B, the peptide 11 (array number 13 of an array table) has the structure where Arg was added to the C terminal of the peptide 12. In order to make Escherichia coli produce these peptide using gene modification technology, DNA (henceforth "h7-3") which encodes the peptide 12 first was prepared so that it might indicate below. If in charge of the design of h7-3, in consideration of the codon usage of Escherichia coli, the restriction enzyme recognition sequence required in order to make h7-3 polymerize behind was added to the five prime end [of a coding region], and three-dash terminal side (BamHI) (BglII), respectively. In consideration of insertion to an expression plasmid, the restriction enzyme SalI recognition sequence was added to the pan of the three-dash terminal side BglII recognition sequence at the three-dash terminal side (array number 52 of an array table).

[0146]First, as a partial sequence of the sense strand of h7-3 to which the restriction enzyme recognition sequence was added, or an antisense strand, It adds to F1 compounded in Example 9 and Example 10, F8–F12, and F15–F18, The further following oligonucleotide: 5'- aaatctatga aagttaccggttgcttcaac cagttcggtc cg-3' (F19, array number 54 of an array table);

5'- agatttccaa gatgccgggt tctggtatgc tgcgatgata ccacgcg-3' (F20, array number 55 of an array table);

5'- gacatcttcg catctaaaaa cttccatctg ca-3' (F21, array number 56 of an array table);

5'- gatgtccgga ccgaactggt tgaaagcaac ggtaactttc at-3' (F22, array number 57 of an array table);

5'- gaaaaacaaa ctgacctctg gtaaaatcgc atcttgc-3' (F23, array number 58 of an array table);

5'- gttttctctgc agatggaagt ttttagatgc gaa-3' (F24, array number 59 of an array table);

It compounded and refined by the method indicated to 1 of Example 9. The five prime end was phosphorylated by the method which indicated F19–F24 of 50pmol to 1 of Example 9 (henceforth "F20p", "F21p", "F22p", "F23p", and "F24p" "F19p").

[0147]Next, F8 p–F 12p which phosphorylated oligonucleotide F1, F18, and a five prime end, F15 p–F 17p and every (1–2microl) 10 pmol each of F19 p–F 24p were mixed, annealing was carried out by a presentation and temperature conditions of Example 9 given in one, and it dissolved in TE buffer solution 10mul after ethanol precipitate. The 5microl was connected using the DNA

ligation kit, and it dissolved in sterile-distilled-water 10mul after ethanol precipitate. [0148]PCR was carried out by using solution 5mul of the connected DNA fragment as a mold using the primers PRv and PFW prepared in Example 10 by a reaction mixture presentation and temperature conditions of Example 10 given in one. After performing isopropanol precipitate about the reaction mixture after PCR, precipitate was collected and low melting point agarose gel electrophoresis was performed 2.5%. The gel of the band part equivalent to the amplified DNA fragment was started, Hiroshi's TE buffer solution was added 4 times, it was kept warm for 5 minutes at 65 **, and gel was dissolved. After the phenol solution of isochore extracted this thing once with phenol chloroform fluid twice at chloroform fluid, ethanol precipitate was performed and precipitate was dissolved in sterile-distilled-water 10mul after washing by ethanol 70%. The DNA fragment which digested this thing (4microl) with the restriction enzyme BamHI, and was further digested by SalI, and pUC18 vector which were prepared by 1 of Example 9 and which carried out BamHI-SalI digestion were connected using the DNA ligation kit. 109 shares of competent Escherichia coli JM were transformed by the method indicated to 1 of Example 9 by this ligation reaction mixture, and it cultivated at 37 **. It isolated, the ampicillin tolerance colony which appeared was inoculated into L-broth culture medium, and shaking culture was carried out at 37 ** overnight. The plasmid pUC (h7-3) containing h7-3 was extracted from 3 ml of this culture medium ([drawing 1](#)). Ethanol precipitate of the extracted plasmid was carried out, and it dissolved in sterile-distilled-water 50mul after 70% ethanol washing. Although t of the nucleotide number 154 of the nucleotide sequence (array number 52 of an array table) designed at the beginning was replaced by c, h7-3 by which cloning was carried out as a result of investigating the nucleotide sequence of h7-3 inserted in pUC (h7-3), Since change was not given to the codon of amino acid Ser (amino acid number 48 of the array number 53 of an array table) by which a code is carried out to the part, it was used for the following examples as it is. The amino acid sequence by which a code is carried out to the nucleotide sequence and this arrangement of h7-3 after substitution was shown in the array numbers 14 and 15 of the array table.

[0149]2) pUC (h7-3) prepared by the preparation above 1 of the peptide 11 was digested with the restriction enzymes BamHI and SalI, and the small fragment (about 310 bp(s)) was refined by the electrophoresis using low melting point agarose gel 2%. On the other hand apart from this, pUC (h7-3) was digested with the restriction enzymes BglII and SalI, and the large fragment (about 3.0 kbp(s)) was refined by low melting point agarose gel electrophoresis 1.0%. Next, plasmid pUC(h7-3)₂ including the nucleotide sequence (array number 60 of an array table) which encodes the amino acid sequence shown in the array number 61 of an array table was obtained by connecting these fragments using a DNA ligation kit ([drawing 1](#)).

[0150]21 shares of competent Escherichia coli YA were transformed by pUC(h7-3)₂ like 3 of Example 9 (even 109 shares of JM are possible). This transformant was inoculated into 2 liter-capacity Erlenmeyer flask into which a 400-ml 2xTY culture medium was put, and rotary shaking culture was carried out at 37 ** for 8 hours. The whole quantity of the obtained culture medium ($OD_{660nm}=2.5$) was moved to the 200 liter-capacity cultivation tank into which a 120-l. 2xTY culture medium was put, further, aeration stirring conditions were changed and culture was continued so that dissolved oxygen concentration might be maintained to 5 ppm at 37 ** for 17 hours.

[0151]After the end of culture, culture medium ($OD_{600}=8.6$) was adjusted the pH to 3.0 with sulfuric acid 15% (v/v), and was returned to pH 7.0 by sodium hydroxide 20% (w/v) after 30-minute stirring. After obtaining a biomass concentrate by membrane filtration (a membranous aperture: 0.2 micrometer), centrifugal separation was performed, precipitate was collected and the wet fungus body 580g was obtained. 20mM TES buffer solution (20mM tris-chloride (pH 7.6).) 5 l. of biomass suspension (the concentration of polymerization protein in SDS-PAGE analysis is about 20%) is adjusted by 150mM sodium chloride and 10mMEDTA, After passing a cell homogenizer (made by an APV gauhin company) for this thing 4 times (50Mpa) and crushing a biomass, 50mM which contains the triton X-100 (final concentration) 0.4% Tris-chloride buffer

solution (pH 7.6) and 50mM By washing and centrifuging with tris-chloride buffer solution, 2.5 l. of slurries (the concentration of polymerization protein in SDS-PAGE analysis is about 33%) were obtained. While sodium hydroxide solution adjusted pH in the solution which added 5 l. of 10M urea solution in this slurry whole quantity 25% (w/v) 8.7, 4.8 ml of anhydrous citraconic acid was added 8 times every 15 minutes. 7.5-l. 20mM calcium nitrate and 264 mg of trypsin were added after adjusting this end liquid of SHITORAKONIRU-ized the pH to 37 ** and 7.6, and it was kept warm at 36 **. 1 hour afterward, 150-ml TFA was added and the reaction was suspended. This reaction mixture was centrifuged and 272 g of precipitate was obtained. [0152]68 g of this precipitate is suspended to 2 l. of 100mM sodium phosphate buffer solution (pH 6.0), After adding 8-l. 10M urea solution and 0.5 l. of beta-mercaptoethanol, it was made to stick to 2 l. of cation exchange resin (SP-sepharose FF, Pharmacia manufacture). It is 8M about resin. By urea / 20mM sodium phosphate (pH 6.0) / 10mM DTT, after washing, It was eluted one by one by the eluate (8M urea, 20mM sodium phosphate and 10mM DTT, pH 6) containing the sodium chloride of 0.05M, 0.1M, 0.2M, 0.3M, and 2.0M. Since target peptide was contained in the eluate fraction of 0.2M sodium chloride concentration, after diluting this fraction with pure water twice, it centrifuged by having added sodium chloride so that it might become the final concentration 3M, and precipitate was collected. After adding pure water to precipitate and being suspended, centrifuge, collect supernatant liquid and Acetonitrile, :column which performed reversed-phase-chromatography refining on condition of the following after adding TFA so that it might become 20% of final concentration, and 0.1%, respectively: ODS column (the YMC-pack ODS, phi10cmx50cm, product made by YMC)

Mobile phase: 20-60% acetonitrile / 0.1% TFA, 65 minutes (linear density gradient)

rate-of-flow: — a part for 260-ml/— detection wave length: — about the substance obtained by isolating a neighboring peak preparatively for 230-nm elution time 44.3 minutes, as a result of conducting amino acid sequence analysis from an amino terminal to 20 residue, it was in agreement with the amino acid sequence shown in the amino acid numbers 1-20 of the array number 13 of an array table. 650 mg of peptide 11 was obtained as mentioned above.

[0153]3) Vector pUC18M5 for a direct manifestation for preparing the preparation peptide 12 of the peptide 12, g of the nucleotide number 177 of the nucleotide sequence (GenBank Accession No.L08752) of pUC18 vector is replaced by c, A lac promotor is changed into lacUV5 promotor by producing pUC18M vector which has a cleavage site of the restriction enzyme MunI (drawing 4), and replacing gt of the nucleotide numbers 169-170 by aa further. Introduction of these variation was performed using the RT-PCR assay (Douglas, H.et al. BioTechniques, 8, and 178 (1990)) (drawing 3).

[0154]Oligonucleotide:5'- tccggctcgt atgttgtgtg caattgtgag c-3' first indicated below as a primer (P1, array number 62 of an array table);

5'- agcataaagt gtaaacctg gg-3' (P2, array number 63 of an array table);

5'- attgttatcc gctcacaatt gcacacaaca ta-3'(P3, array number 64 of array table); and 5'-

ttcacacagg aaacagctat gacc-3' (P4, array number 65 of an array table)

It compounded.

[0155]Next, PCR was carried out with the following monograph affairs :P CR (1)

Reaction mixture presentation: Template DNA pUC18 2ng. 10 pmol/mu l Primer P1 2.5microl 10 pmol/mu l Primer P2 2.5microl 10 time concentration Ex Taq PCR buffer solution 5microl dNTP mixed liquor 4microl Ex Taq Polymerase 0.5 unit The whole quantity was set to 50microl with sterile distilled water.

Temperature conditions: After having repeated at 50 **, heating for 1 minute at 94 ** and repeating the temperature cycle for 2.5 minutes 30 times at 72 ** for 30 seconds for 30 seconds at 94 **, it was kept warm for 7 minutes at 72 **.

[0156]

PCR(2)

Reaction mixture presentation: Mold pUC18 2ng. 10 pmol/mu l Primer P3 2.5microl10 pmol/mu l Primer P4 2.5microl 10 time concentration Ex Taq PCR buffer solution 5microl dNTP mixed liquor 4microl Ex Taq Polymerase 0.5 unit The whole quantity was set to 50microl with sterile distilled water.

Temperature conditions: It is the same as above-mentioned PCR (1).

[0157]Isopropanol precipitate of PCR (1) and the reaction product of (2) was carried out, respectively, and it dissolved in sterile-distilled-water 20μl. :reaction mixture presentation which mixed each product and performed annealing operation on condition of the following (0.1M tris-chloride buffer solution (pH 8.0) — 10 mM EDTA) Reaction product of PCR (1) Reaction product of 1μmol PCR (2) 1μmol 10 time concentration Annealing buffer solution 1M sodium chloride — the whole quantity was set to 50μmol with 5μmol sterile distilled water.

Temperature conditions: It heated for 3 minutes at 94 **, and cooled to the room temperature (25 **) after 2-hour incubation at 50 **.

[0158]Subsequently, 109 shares of competent Escherichia coli JM are transformed by the method of Example 9 given in one using 5μmol of reaction mixture I after annealing, It applied to L-broth agar medium containing the 5-bromo-4-chloro-3-indolyl beta-D-galactopyranoside (henceforth "X-gal") of the isopropylthio galactopyranoside (henceforth "IPTG") of 200μmol, and 800μmol, extended, and cultivated at 37 **. The blue colony which appeared was isolated, it cultivated overnight by L-broth culture medium containing 50 μmol/ml ampicillin, and the plasmid was extracted from culture medium. It was checked with one band having appeared in the position of about 2.8 kbp(s) whether a MunI recognition site exists in this plasmid, as a result of digesting and carrying out agarose gel electrophoresis of the plasmid 1% with the restriction enzyme MunI. The nucleotide sequence of the part which introduced variation was also checked. Since the transformant holding this plasmid formed the blue colony on L-broth agar medium containing IPTG and X-gal, it was shown that the LacZ gene after substitution is functioning normally.

[0159]Next, as a primer for introducing the variation which changes a lac promotor into lacUV5 promotor by using pUC18M vector as a mold, The oligonucleotide indicated below: 5'- tttatgcttc cggctcgat aatgtgtgca at-3' (P21, array number 66 of an array table);

5'- gtgtaaagcc tgggtgcct aa-3' (P22, array number 67 of an array table);

5'- tccgctcaca attgcacaca ttatacgagc cg-3'(P23, array number 68 of array table); and 5'- taacaatttc acacaggaaa cag-3' (P24, array number 69 of an array table)

It compounded and PCR was carried out on condition of the following :P CR (3)

Reaction mixture presentation: Mold pUC18M 10ng. 10 pmol/μmol I Primer P21 2.5μmol 10 pmol/μmol I Primer P22 2.5μmol 10 time concentration Ex Taq PCR buffer solution 5μmol dNTP mixed liquor 4μmol Ex Taq Polymerase 1.25 units With sterile distilled water, the whole quantity. It was referred to as 50μmol.

Temperature conditions: After having repeated at 55 **, heating for 3 minutes at 94 ** and repeating the temperature cycle for 3 minutes 30 times at 72 ** for 30 seconds for 30 seconds at 94 **, it was kept warm for 10 minutes at 72 **.

[0160]

PCR(4)

Reaction mixture presentation: Mold pUC18M 10ng. 10 pmol/μmol I Primer P23 2.5μmol 10 pmol/μmol I Primer P24 2.5μmol 10 time concentration Ex Taq PCR buffer solution 5μmol dNTP mixed liquor 4μmol Ex Taq Polymerase 1.25 units With sterile distilled water, the whole quantity. It was referred to as 50μmol.

Temperature conditions: It is the same as above-mentioned PCR (3).

[0161]Carry out isopropanol precipitate of the reaction product after each PCR, and it dissolves in sterile-distilled-water 20μl after washing by ethanol 70%, respectively, Annealing buffer solution of the :reaction mixture presentation:10 time concentration to which annealing of both was carried out on condition of the following Reaction product of 5μmolPCR (3) Reaction product of 1μmolPCR (4) The whole quantity was set to 50μmol with 1μmol sterile distilled water.

Temperature conditions: It heated for 3 minutes at 94 **, and cooled to the room temperature (25 **) after 2-hour incubation at 50 **.

[0162]Subsequently, by the method of Example 9 given in one, 109 shares of competent Escherichia coli JM were transformed using 5μmol of reaction mixture I after annealing, and it applied to L-broth agar medium containing IPTG of 200μmol, and X-gal of 800μmol, extended,

and cultivated at 37 **. The blue colony which appeared was isolated, it cultivated overnight by L-broth culture medium containing 50 microg/ml ampicillin, and the plasmid was extracted. It was checked by analyzing the nucleotide sequence of the obtained plasmid that the target variation is introduced. Since the transformant holding this plasmid formed the blue colony on L-broth agar medium containing IPTG and X-gal, it was shown that lacUV5 promotor newly made by variation introduction is functioning normally. By the above operation, vector pUC18M5 (array number 70 of an array table) for a direct manifestation for preparing the peptide 12 were obtained.

[0163] Subsequently, h7-3 obtained by the above 1 as a primer for PCR for connecting immediately after the initiation codon (atg) by the side of the three-dash terminal of lacUV5 promotor of pUC18M5 vector, The oligonucleotide indicated below: 5'- ggtatcatcg cagcatacca gaa-3' (P101, array number 71 of an array table);

5'- ttaacccatc aggggtgaaac cg-3' (P102, array number 72 of an array table);

5'- gataacaatt tcacacagga aacagctatgggtatcatcg cagcatacca gaa-3' (P103, array number 73 of an array table);

5'- agaggatccc cgggtaccga gctcgaattc ttaacccatc aggggtgaaac cg-3' (P104, array number 74 of an array table);

5'- gaattcgagc tcggtacccg gg-3' (P105, array number 75 of an array table);

5'- catagctgtt tcctgtgtga aa-3' (P106, array number 76 of an array table);

5' --- ' - agagtcgacc tgcagggcatg ca-3' (P107, array number 77 of array table); and 5'- cgctcacaat tgcacacatt at-3' (P108, array number 78 of an array table)

It compounded and PCR was carried out on condition of the following :P CR (5)

Reaction mixture presentation: Template DNA pUC (h7-3). 200ng 10 pmol/mu l primer . P101 2.5microl 10 pmol/mu l Primer P102 2.5microl 10 time concentration Ex Taq PCR buffer solution 5microl dNTP mixed liquor 4microl Ex Taq 1.25 units of polymerases The whole quantity was set to 50microl with sterile distilled water.

Temperature conditions: After having repeated at 55 **, heating for 3 minutes at 94 ** and repeating the temperature cycle for 3 minutes 30 times at 72 ** for 30 seconds for 30 seconds at 94 **, it was kept warm for 10 minutes at 72 **.

[0164]

PCR(6)

Reaction mixture presentation: Template DNA pUC (h7-3). 200ng 10 pmol/mu l primer . P103 2.5microl 10 pmol/mu l Primer P104 2.5microl 10 time concentration Ex Taq PCR buffer solution 5microl dNTP mixed liquor 4microl Ex Taq 1.25 units of polymerases The whole quantity was set to 50microl with sterile distilled water.

Temperature conditions: It is the same as above-mentioned PCR (5).

[0165]

PCR(7)

Reaction mixture presentation: Template DNA solution pUC18M5. 10ng 10 pmol/mu l The primer P105. 2.5microl 10 pmol/mu l Primer P106 2.5microl 10 time concentration Ex Taq PCR buffer solution 5microl dNTP mixed liquor 4microl Ex Taq 1.25 units of polymerases The whole quantity was set to 50microl with sterile distilled water.

Temperature conditions: It is the same as above-mentioned PCR (5).

[0166]

PCR(8)

Reaction mixture presentation: Template DNA solution pUC18M5. 10ng 10 pmol/mu l The primer P107. 2.5microl 10 pmol/mu l Primer P108 2.5microl 10 time concentration Ex Taq PCR buffer solution 5microl dNTP mixed liquor 4microl Ex Taq Polymerase 1.25 units The whole quantity was set to 50microl with sterile distilled water.

Temperature conditions: It is the same as above-mentioned PCR (5).

[0167] Isopropanol precipitate was carried out about the reaction mixture after each PCR, and it dissolved in the sterile distilled water of 20microl after 70% ethanol washing, respectively. The 5microeach l was taken and low melting point agarose gel electrophoresis was performed 2%. The gel of the band part equivalent to the amplified DNA fragment was started, Hiroshi's TE buffer

solution was added 3 times, it was kept warm for 5 minutes at 65 **, and gel was dissolved. After the phenol solution of isochore extracted this thing once with phenol chloroform fluid twice at chloroform fluid, ethanol precipitate was performed and precipitate was dissolved in sterile-distilled-water 10mul after washing by ethanol 70%, respectively. :reaction mixture presentation which mixed these and performed annealing on condition of the following: PCR (5) Each reaction product solution of - (8) 1microeach l 10 time concentration Annealing buffer solution 10microl The whole quantity was set to 100microl with sterile distilled water.

Temperature conditions: It heated for 3 minutes at 94 **, and cooled to the room temperature (25 **) after 2-hour incubation at 50 **.

[0168]Subsequently, by the method of Example 9 given in one, 109 shares of competent Escherichia coli JM were transformed using 10micro of reaction mixture l after annealing, and it applied to L-broth agar medium containing IPTG of 200microg, and X-gal of 800microg, extended, and cultivated at 37 **. The white colony which appeared was isolated, it cultivated overnight by L-broth culture medium containing 50 microg/ml ampicillin, and the plasmid was extracted. It was checked by investigating the nucleotide sequence of an insert whether the target plasmid had been obtained. Plasmid pUCM5-h73 was obtained as mentioned above.

[0169]Next, this pUCM5-h73 was digested with the restriction enzymes MunI and SalI, low melting point agarose gel electrophoresis was performed, and that small fragment (about 360 bp (s)) was extracted from gel. On the other hand apart from this, pUCM5-h73 was digested with the restriction enzymes EcoRI and SalI, low melting point agarose gel electrophoresis was performed, and the large fragment (about 2.9 kbp(s)) was extracted from gel. Plasmid pUCM5-2h73 was obtained by connecting these two fragments using a DNA ligation kit (drawing 6).

[0170]Similarly by connecting the MunI-SalI digestive smallness fragment (about 700 bp(s)) of this pUCM5-2h73, and an EcoRI-SalI digestive large fragment (about 3.3 kbp(s)), Obtain plasmid pUCM5-4h73 and further The MunI-SalI digestive smallness fragment (about 690 bp(s)) of pUCM5-2h73, Plasmid pUCM5-6h73 was obtained by connecting the EcoRI-SalI digestive large fragment (about 3.9 kbp(s)) of pUCM5-4h73 (drawing 6). The unit of [ribosome coupling region +h7-3] of many copies in this pUCM5-6h73 (array number 79 of an array table) is directly connected by each immediately after the initiation codon by the side of the three-dash terminal of lacUV5 promotor of the pUC18M5 origin. The peptide 12 can be made to produce directly and efficiently by introducing these plasmids into Escherichia coli.

[0171]21 shares of competent Escherichia coli YA were transformed by pUCM5-6h73 of 5ng like 3 of Example 9 (even 109 shares of JM are possible). Inoculation of this transformant was carried out to 100 ml of 2xTY culture media containing 100 microg/ml ampicillin, and shaking culture was performed at 37 ** for 14 hours. 45 ml of culture medium after the end of culture was taken, 6000xg, 4 **, and the biomasses that performed centrifugal separation for 20 minutes and precipitated were collected, and the biomass was crushed with the ultrasonic homogenizer after being suspended to 4.5 ml of TES buffer solution. After having centrifuged this thing for 20 minutes at 13000xg and 4 **, collecting precipitate and repeating washing with TES buffer solution 3 times further, a 4.5-ml 0.75% N-lauryl sarcosine solution washed twice with distilled water further 4 times (centrifugal-separation conditions: for 25000xg, 4 **, and 20 minutes). It solubilized having been suspended to 4.5-ml 6M guanidine solution, and shaking the obtained precipitate at 37 **. 1 hour afterward, it centrifuged for 20 minutes at 25000xg and 4 **, supernatant liquid was collected, and it was considered as the rough refining liquid of the peptide 12.

[0172]:column:TSK-gel G-3000SW_{XL} (phi -- 7.8x300 mm) which performed gel-filtration-chromatography refining on condition of the following about 1 ml of the above-mentioned rough refining liquid solvent by TOSOH [CORP.] CORP.: -- 30% acetonitrile rate-of-flow: which contains TFA 0.1% -- a part for 0.5-ml/-- detection wave length: -- the :column:ODS column (TSK-gel ODS-120T) which collected neighboring peaks for 280-nm retention time 20.2 minutes, and performed opposite phase HPLC refining further on condition of the following Mobile phase: 36-43% acetonitrile / 0.1% TFA, 35 minutes (linear density gradient) rate-of-flow: -- a part for 2-ml/-- detection wave length: -- as a result of isolating a

neighboring peak preparatively for 220-nm retention time 13.4 minutes and conducting amino acid sequence analysis to amino terminal 10 residue, it was in agreement with the amino acid sequence shown in the amino acid numbers 1-10 of the array number 15 of an array table. As a result of measuring a molecular weight by the ESI method, the calculative molecular weight of the peptide 12 and the molecular weight of the substance mainly contained in this peak were in agreement. About 6 mg per 100 ml of culture medium of peptide 12 was able to be obtained as mentioned above.

[0173][Reference example] pUC (h6-1), pUC (h7-1), or pUC (h7-3) produced in Examples 9-11, It digested with the restriction enzymes BamHI and Sall, and those small fragments (respectively about 270 bp(s), about 300 bp(s), or about 310 bp(s)) were refined by agarose gel electrophoresis. On the other hand, cloning vector pBR322 was digested with the restriction enzymes BamHI and Sall, and the large fragment (about 4.1 kbp(s)) was refined by agarose gel electrophoresis. The plasmid pBR (h6-1) which connected and obtained these DNA fragments, pBR (h7-1) or pBR (h7-3) is introduced into 109 shares of competent Escherichia coli JM. Transformation Escherichia coli E.coli pBR(h6-1) SANK 70199, E.coli pBR(h7-1) SANK 70299, and E.coli pBR(h7-3) SANK 70399 which were obtained, It ****ed to National Institute of Bioscience and Human-Technology, the Agency of Industrial Science and Technology, on year February 9 in Heisei 10 (1998), and accession number FERM BP-6642, FERM BP-6643, and FERM BP-6644 were attached, respectively.

[0174][Example 12] It checked using the T cell of cedar pollen antigen T cell epitope activity hay fever patient origin by the method of indicating the cedar pollen antigen T cell epitope activity of the peptide 1 thru/ or 12 of this invention below. 50 ml of peripheral blood was extracted from the patient who shows hay fever condition. peripheral blood — the Hanks balanced salt solution (Hanks' —) [Balanced Salt Solution and] After diluting with HBSS, fractionation of the peripheral mononuclear blood cell (Peripheral Blood Mononuclear Cells and henceforth "PBMC") was carried out by the ficoll pack specific gravity centrifuge method, and it was suspended to the culture medium (RPMI1640 containing 5% of Homo sapiens AB type blood serum).

[0175]The cell of the 4×10^5 individual was poured distributively per one well on the flat bottom plate of 96 holes. The peptide 9 or 10 was added so that the peptide 4, 5, 6, 11, or 12 or the last concentration might serve as 50nM, as the last concentration became each well in ml and 1 microg /simultaneously and the peptide 1, 2, 3, 7, or 8 or the last concentration served as 100nM. The group which adds no peptide was prepared as a negative control. The cell was cultured under 37 ** and 5% carbon dioxide in the culture medium of 200microl for 72 hours. Then, 0.5 microcurie of tritition thymidine was added and it cultivated for further 16 hours. The cells of each well were collected on the glass fiber filter using the cell harvester, and the tritition thymidine content taken into the cell with the liquid scintillation counter, respectively was measured. The numerical value which *(ed) incorporation thymidine content with the uptake quantity of the negative control group was made into the T cell stimulation index, and the group to which this T cell stimulation index reached the value exceeding 2 was made into T cell epitope activity "positivity." This result is shown in Table 1.

[0176]

[Table 1]

ペプチド	T細胞エпитープ活性
ペプチド 1	陽性
ペプチド 2	陽性
ペプチド 3	陽性
ペプチド 4	陽性
ペプチド 5	陽性
ペプチド 6	陽性
ペプチド 7	陽性
ペプチド 8	陽性
ペプチド 9	陽性
ペプチド 10	陽性
ペプチド 11	陽性
ペプチド 12	陽性

[0177]Each peptide prepared in Examples 1 thru/ or 11 showed T cell epitope activity

"positivity."

[Example 13] By having connected synergistic effect T cell epitope peptide by connection, it was shown by the following methods that the activity sthenia with the synergistic T cell epitope activity of connection peptide is shown.

[0178]First, following peptide:Gly-Ile-Ile-Ala-Ala-Tyr-Gln-Asn-Pro-Ala-Ser-Trp which is used as contrast and which consists of an independent T cell epitope which is not connected (fairly to the peptide 13 and a T cell epitope "A") Array number 16 of an array table;

Gln-Phe-Ala-Lys-Leu-Thr-Gly-Phe-Thr-Leu-Met-Gly (it is the array number 17 of considerable and an array table to the peptide 14 and a T cell epitope "B");

Phe-Ala-Ser-Lys-Asn-Phe-His-Leu-Gln-Lys-Asn-Thr (it is the array number 18 of considerable and an array table to the peptide 15 and a T cell epitope "C");

Lys-Leu-Thr-Ser-Gly-Lys-Ile-Ala-Ser-Cys-Leu-Asn (it is the array number 19 of considerable and an array table to the peptide 16 and a T cell epitope "D");

Ser-Met-Lys-Val-Thr-Val-Ala-Phe-Asn-Gln-Phe-Gly-Pro (it is the array number 20 of considerable and an array table to the peptide 17 and a T cell epitope "E");

Met-Lys-Val-Thr-Val-Ala-Phe-Asn-Gln-Phe-Gly (it is the array number 21 of considerable and an array table to the peptide 18 and a T cell epitope "E'");

Tyr-Gly-Leu-Val-His-Val-Ala-Asn-Asn-Asn-Tyr-Asp-Pro (fairly to the peptide 19 and a T cell epitope "F") Array number 22; and Ser-Gly-Lys-Tyr-Glu-Gly-Gly-Asn-Ile-Tyr-Thr-Lys-Lys-Glu-Ala-Phe-Asn-Val-Glu (it is the array number 23 of considerable and an array table to the peptide 20 and a T cell epitope "G") of an array table

It compounded by the same method as example 7 statement.

[0179]After, extracting 50 ml of peripheral blood on the other hand from the patient who shows hay fever condition and diluting with HBSS, fractionation of the PBMC was carried out by the ficoll pack specific gravity centrifuge method, and it was suspended to the culture medium (RPMI1640 containing 5% of Homo sapiens AB type blood serum). The cell of the 4×10^5 individual was poured distributively per one well on the flat bottom plate of 96 holes, and it added so that the last concentration might serve as 10pM thru/or 100nM simultaneously at each well in the peptide 1, 2, 3, 4, 5, 6, 11, or 12. About the peptide 9 and the peptide 10, to each well, the peptide 9 or 10 was added so that the last concentration might serve as 5pM thru/or 50nM. The following four groups were provided as a peptide mixed culture group. Namely, the group added so that each of the peptide 13, 15, 16, 17, 19, and 20 might serve as last concentration 10pM thru/or 100nM per one well, The group added so that each of the peptide 13, 15, 16, 18, 19, and 20 might serve as last concentration 10pM thru/or 100nM per one well, The group added so that each of the peptide 13, 14, 15, 16, 17, 19, and 20 might serve as last concentration 10pM thru/or 100nM per one well, The group added so that each of the peptide 7, 8, 14, 16, 17, 19, and 20 might serve as 100nM from last concentration 10pM per one well was provided. Thus, the cell which carried out conditioning was cultured under 37 ** and 5% carbon dioxide in the culture medium of 200microl for 72 hours. Then, 0.5 microcurie of tritition thymidine was added and it cultivated for further 16 hours. The cells of each well were collected on the glass fiber filter using the cell harvester, and the tritition thymidine content taken into the cell with the liquid scintillation counter, respectively was measured. And about the peptide 1 or a peptide 9 addition group, the composition of these and a T cell epitope compared the mixed culture group and T cell epitope activity of the peptide 13, 15, 16, 17, 19, and 20 which become common (drawing 7). About the peptide 2 or a peptide 3 addition group, the mixed culture group and T cell epitope activity of the peptide 13, 15, 16, 18, 19, and 20 were compared similarly (drawing 8). About the peptide 4 or a peptide 10 addition group, the mixed culture group and T cell epitope activity of the peptide 13, 14, 15, 16, 17, 19, and 20 were compared similarly (drawing 9). About the peptide 5, the peptide 6, the peptide 11, or a peptide 12 addition group, the mixed culture group and T cell epitope activity of the peptide 7, 8, 14, 16, 17, 19, and 20 were compared similarly (drawing 10, drawing 11). However, about the result of the peptide 9 and a peptide 10 addition group, in order to amend that each two T cell epitopes of every are contained in this peptide, it plotted in the position twice the concentration of actual molar concentration.

[0180]As a result, it was shown at least 10 times rather than the case where the direction at the time of [with same total amount as an added T cell each epitope] connecting them but was only mixed, and adds that the activity as a T cell epitope becomes high.

[0181][Example 14] It is targeted at 114 hay fever patients who reached the value exceeding the T cell stimulation index 2 by the reactivity of the T cell which receives any of the importance cedar pollen allergen Cryj1 or Cryj2 of T cell epitope peptide they are, The T cell epitope activity of the peptide 7 and the peptide 8 was evaluated in accordance with the method of a statement in the Example 12, and it asked for the importance index. An importance index (Positivity Index) is the numerical value which multiplied the an average of T cell stimulation index by positive frequency, and it is indicated in WO 94/No. 01560 gazette. The geometric mean of the T cell stimulation index about the test subject the an average of T cell stimulation index indicated the positive reaction to be to this peptide, and positive frequency express the rate (percentage) of the test subject who showed the positive reaction to this peptide, respectively. A result is shown in Table 2.

[0182]

[Table 2]

ペプチド	平均T細胞刺激指数	陽性頻度	重要度指数
ペプチド7	5.52	57%	315
ペプチド8	4.62	54%	247

[0183]Thus, the peptide 7 and the peptide 8 all showed the high importance index among hay fever patients, and it was shown that it is useful as peptide for desensitization therapies to hay fever.

[0184][Example 1 of pharmaceutical preparation] The peptide obtained by the method given in Examples 1 thru/or 11 to the physiological saline which contains a human serum albumin 1% (w/v) as injections stabilizer is dissolved so that it may become in the last concentration 0.01, 0.1, or ml and 1mg /, After carrying out filtration sterilization, 2 ml is poured distributively to each sterilization vial bottle, and it freeze-dries and seals to it. In advance of administration, this article adds 1 ml of distilled water for injection etc. in a vial bottle first, and subsequently it uses contents, dissolving uniformly. This article which is excellent in stability and contains peptide by this invention as an active principle is useful as dry pharmaceutical preparation for treating and preventing hay fever.

[0185][Example 2 of pharmaceutical preparation] So that it may become in 0.1mg/ml about either of the peptide obtained by the method given in the syrups examples 1 thru/or 11 and may become 50% (w/v) about trehalose powder (TOREHAOSU, Hayashibara Biochemical Laboratories Make), It dissolves in distilled water, respectively, sterilization filtration of the solution is carried out with a conventional method, and 12 kinds of sirupy things are obtained. These sirupy things are independently poured distributively to a sterilization vial bottle, respectively, and it seals them 2 ml at a time to it. This article which is excellent in stability and is easy to take in is useful as syrups for treating and preventing hay fever.

[0186]a [acute toxicity test] — the pharmaceutical preparation obtained to the day [of after the birth / 20th] mouse by the method given in the example 1 or 2 of pharmaceutical preparation — a conventional method — taking orally — or abdominal administration is carried out. fifty percent lethal dose of a specimen is more than 200 mg/kg mouse weight by any route of administration (as peptide).

This shows that peptide of this invention can carry out combination use to the anti-hay fever agent with which the mammals including Homo sapiens are medicated safely.

[0187]

[Effect of the Invention]The anti-hay fever agent which contains peptide and them which consist of a T cell epitope of cedar pollen allergen as an active principle by this invention was able to be provided. And peptide of this invention can inactivate a specific T cell to cedar pollen allergen, without causing anaphylaxis substantially, if the general mammals including Homo sapiens are

medicated since it does not react to an immunoglobulin E antibody specific to cedar pollen allergen substantially. And peptide of this invention containing 6 or seven T cell epitopes which are different in the intramolecular has activity by a low dose, and can lessen the dose to a patient rather than mixing and prescribing each epitope for the patient. The anti-hay fever agent which contains peptide of this invention as an active principle is made into a low dose, and is effective to a more extensive hay fever patient.

[0188]

[Layout Table]

SEQUENCE LISTING <110> Kabushiki Kaisha Hayashibara Seibutsu Kagaku Kenkyujo Sankyo Company, Limited <120> Peptides and The Uses Thereof <130> P00-0129 <150> JP99/68316<151> 1999-3-15 <160> 80 <170> PatentIn Ver. 2.0 <210> 1<211> 81<212> PRT<213> Artificial Sequence <220> <223> Description of Artificial Sequence: Designed peptide which consists of T cell epitopes derived from cedar pollen allergens -- <400> 1Gly Ile Ile Ala Ala Tyr Gln Asn Pro Ala Ser Trp Ser Met Lys Val 1 5 10 15 Thr Val Ala Phe Asn Gln Phe Gly Pro Phe Ala Ser Lys Asn Phe His. 20 25 30 Leu Gln Lys. Asn Thr Lys Leu Thr. Ser Gly Lys Ile Ala Ser Cys Leu 35 40 45 Asn Ser Gly Lys Tyr Glu Gly Gly Asn Ile Tyr Thr Lys Lys Glu Ala 50 55 60 Phe Asn Val Glu Tyr Gly Leu Val His Val Ala Asn Asn Asn Tyr Asp 65 70 75 80 Pro <210>2<211> 79<212> PRT<213> Artificial Sequence. <220> <223> Description. of Artificial Sequence:. Designed peptide which consists of T cell epitopes. derived from cedar pollen. allergens <400> 2Gly. Ile Ile Ala Ala Tyr. Gln Asn Pro Ala Ser. Trp Met Lys Val Thr. 1 5 10 15 Val Ala Phe. Asn Gln Phe Gly Phe. Ala Ser Lys Asn Phe His Leu Gln 20 25 30 Lys Asn Thr Lys Leu Thr Ser Gly Lys Ile Ala Ser Cys Leu Asn Tyr 35. 40 45 Gly Leu Val His. Val Ala Asn Asn Asn. Tyr Asp Pro Ser Gly Lys Tyr 50 55 60 Glu Gly Gly Asn Ile Tyr Thr Lys Lys Glu Ala Phe Asn Val Glu 65 70 75 -- <210> 3<211> 79<212> PRT<213> Artificial Sequence <220> <223> Description of Artificial Sequence:Designed peptide which consists of T cell epitopes. derived from cedar pollen. allergens <400> 3Gly. Ile Ile Ala Ala Tyr. Gln Asn Pro Ala Ser Trp Met Lys Val Thr 1 5 10 15 Val Ala Phe Asn Gln Phe Gly Phe Ala Ser Lys Asn Phe His Leu Gln 20 25 30 Lys Asn Thr Lys Leu Thr Ser Gly Lys Ile Ala Ser Cys Leu Asn Ser 35 40 45 Gly Lys Tyr Glu Gly Gly Asn Ile Tyr Thr Lys Lys Glu Ala Phe Asn 50 55 60 Val Glu Tyr Gly Leu Val His Val Ala Asn Asn Asn Tyr Asp Pro 65 70 75 -- <210> 4<211> 93<212> PRT<213> Artificial Sequence <220> <223> Description of Artificial Sequence:Designed peptide which consists of T cell epitopes. derived from cedar pollen. allergens <400> 4Gln. Phe Ala Lys Leu Thr. Gly Phe Thr Leu Met. Gly Gly Ile Ile Ala. 1 5 10 15 Ala Tyr Gln. Asn Pro Ala Ser Trp. Ser Met Lys Val Thr. Val Ala Phe 20 25 30. Asn Gln Phe Gly Pro. Phe Ala Ser Lys Asn. Phe His Leu Gln Lys Asn 35 40 45 Thr Lys Leu Thr Ser Gly Lys Ile Ala Ser Cys Leu Asn Tyr Gly Leu 50 55 60 Val His Val. Ala Asn Asn Asn Tyr Asp Pro Ser Gly Lys Tyr Glu Gly 65 70 75 80 Gly Asn Ile Tyr Thr Lys Lys Glu Ala Phe Asn Val Glu 85 90 -- <210> 5<211> 95<212> PRT<213> Artificial Sequence <220> <223> Description of Artificial Sequence:Designed peptide which consists of T cell epitopes. derived from cedar pollen allergens <400> 5Gln Phe Ala Lys Leu Thr Gly Phe Thr Leu Met Gly Gly Ile Ile Ala 1 5 10 15 Ala Tyr Gln Asn Pro Ala Ser Trp Lys Ser Met Lys Val Thr Val Ala 20 25 30 Phe Asn Gln Phe Gly Pro Asp Ile Phe Ala Ser Lys Asn Phe His. Leu 35 40 45 Gln Lys. Asn Lys Leu Thr Ser. Gly Lys Ile Ala Ser. Cys Leu Asn Tyr 50 55. 60 Gly Leu Val His Val. Ala Asn Asn Asn Tyr. Asp Pro Ser Gly Lys Tyr 65 70 75 80 Glu Gly Gly Asn Ile Tyr Thr Lys Lys Glu Ala Phe Asn Val Glu 85 90 95 -- <210> 6<211> 99<212> PRT<213> Artificial Sequence <220> <223> Description of Artificial Sequence:Designed peptide which comprises T cell epitopes. derived from cedar pollen. allergens <400> 6Gly. Asp Pro Arg Gln Phe. Ala Lys Leu Thr Gly. Phe Thr Leu Met Gly. 1 5 10 15 Gly Ile Ile. Ala Ala Tyr Gln Asn. Pro Ala Ser Trp Lys. Ser Met Lys 20 25 30. Val Thr Val Ala Phe. Asn Gln Phe Gly Pro. Asp Ile Phe Ala Ser Lys 35 40 45 Asn Phe His Leu Gln Lys Asn Lys Leu Thr Ser Gly Lys Ile Ala Ser 50 55 60 Cys Leu Asn Ty. r Gly Leu Val His Val. Ala Asn Asn Asn Tyr. Asp Pro 65 70 75 80. Ser Gly Lys Tyr Glu. Gly Gly Asn Ile Tyr. Thr Lys Lys Glu Ala. Phe 85 90 95 Asn Val. Glu. <210> 7<211> 13 <212> PRT<213> Cryptomeria japonica <400> 7Gly Ile Ile Ala Ala Tyr Gln Asn Pro Ala Ser Trp Lys 1 5 10 <210> 8<211> 13<212> PRT<213> Cryptomeria japonica<400> 8Asp Ile Phe Ala Ser Lys Asn Phe His Leu Gln Lys Asn 1 5 10 <210> 9<211> 558<212> DNA<213> Artificial Sequence <220> <221> CDS<222> (1)..(558) <220> <221>

mat#peptide<222> (4)..(558) <220> <223>Description of Artificial Sequence: Designed sequence encoding a polypeptide which comprises T cell epitopes derived from cedar pollen allergens -- < 400> 9atg acc atg att acg aat tcg agc tcg. gta ccc ggg gat ccg. cgt ggt 48Met Thr Met. Ile Thr Asn Ser Ser. Ser Val Pro Gly Asp. Pro Arg Gly-1 1 5 10. 15 atc atc gca gca tac. cag aac ccg gca tct. tgg tct atg aaa gtt. acc 96Ile Ile Ala Ala. Tyr Gln Asn Pro Ala. Ser Trp Ser Met Lys. Val Thr 20 25 30 gtt. gct ttc aac cag ttc. ggt ccg ttc gca tct. aaa aac ttc cat ctg. 144 Val Ala Phe Asn. Gln Phe Gly Pro Phe. Ala Ser Lys Asn Phe His Leu 35 40 45 cag aaa aac acc aaa ctg acc tct ggt aaa atc gca tct tgc ctg aac 192 G. In Lys Asn Thr Lys Leu Thr Ser Gly Lys Ile Ala Ser Cys Leu Asn 50 55 60 tac ggt ctg gtt cat gtt gca aac aac aac. tac gac ccg tct ggt. aaa 240 Tyr Gly Leu. Val His Val Ala Asn. Asn Asn Tyr Asp Pro. Ser Gly Lys 65 70 75. tac gaa ggt ggt aac. atc tac acc aaa aaa. gaa gca ttc aat gtt. gaa 288 Tyr Glu Gly. Gly Asn Ile Tyr Thr. Lys Lys Glu Ala Phe. Asn Val Glu 80 85 90. 95 cgt gca gat ccg cgt. ggt atc atc gca gca. tac cag aac ccg gca. tct 336 Arg Ala Asp. Pro Arg Gly Ile Ile. Ala Ala Tyr Gln Asn Pro Ala Ser 100 105 110 tgg tct atg aaa gtt acc gtt gct ttc aac cag ttc ggt ccg ttc gca 384 T. rp Ser Met Lys Val Thr. Val Ala Phe Asn Gln. Phe Gly Pro Phe Ala. 115 120 125 tct aaa. aac ttc cat ctg cag. aaa aac acc aaa ctg. acc tct ggt aaa 432. Ser Lys Asn Phe His. Leu Gln Lys Asn Thr. Lys Leu Thr Ser Gly. Lys 130 135 140 atc. gca tct tgc ctg aac. tac ggt ctg gtt cat. gtt gca aac aac aac. 480 Ile Ala Ser Cys. Leu Asn Tyr Gly Leu. Val His Val Ala Asn. Asn Asn 145 150 155. tac gac ccg tct ggt. aaa tac gaa ggt ggt aac atc tac acc aaa aaa 528 Tyr Asp Pro Ser Gly Lys Tyr Glu Gly Gly Asn Ile Tyr Thr Lys Lys 160 1. 65 170 175 gaa gca ttc aat gtt gaa cgt gca gat ctg 558 Glu Ala Phe Asn Val Glu Arg Ala Asp Leu 180 185 -- < 210> 10<211> 186<212> PRT<213> Artificial Sequence <220> <223> Description of Artificial Sequence:Designed peptide which comprises T cell epitopes. derived from cedar pollen. allergens <400> 10Met. Thr Met Ile Thr Asn. Ser Ser Ser Val Pro. Gly Asp Pro Arg Gly. -1 1 5 10 15 Ile Ile. Ala Ala Tyr Gln Asn. Pro Ala Ser Trp Ser Met Lys Val Thr 20 25 30 Val Ala Phe Asn Gln Phe Gly Pro Phe Ala Ser Lys Asn Phe His Leu 35 40 45 Gln Lys Asn Thr Lys Leu Thr Ser. Gly Lys Ile Ala Ser. Cys Leu Asn 50 55 60. Tyr Gly Leu Val His. Val Ala Asn Asn Asn. Tyr Asp Pro Ser Gly. Lys 65 70 75 Tyr Glu. Gly Gly Asn Ile Tyr. Thr Lys Lys Glu Ala. Phe Asn Val Glu 80 85. 90 95 Arg Ala Asp Pro. Arg Gly Ile Ile Ala. Ala Tyr Gln Asn Pro. Ala Ser 100 105 110. Trp Ser Met Lys Val. Thr Val Ala Phe Asn. Gln Phe Gly Pro Phe. Ala 115 120 125 Ser. Lys Asn Phe His Leu Gln Lys Asn Thr Lys Leu Thr Ser Gly Lys 130 135 140 Ile Ala Ser Cys Leu Asn Tyr Gly Leu Val. His Val Ala Asn Asn. Asn 145 150 155 Tyr. Asp Pro Ser Gly Lys. Tyr Glu Gly Gly Asn Ile Tyr Thr Lys Lys 160 165 170 175 Glu Ala Phe Asn Val Glu Arg Ala Asp Leu 180 185 -- < 210> 11<211> 630<212> DNA<213> Artificial Sequence <220> <221> CDS< 222> (1)..(630) <220> <221> mat#peptide<222> (4)..(630) <220> <223>Description of Artificial Sequence: Designed sequence encoding a polypeptide which comprises T cell epitopes derived from cedar pollen allergens -- < 400> 11atg acc atg att acg aat tcg agc tcg gta ccc ggg gat ccg cgt ggt 48Met Thr Met Ile Thr Asn Ser. Ser Ser Val Pro Gly Asp Pro Arg Gly-1 1 5 10 15 atc atc gca gca tac cag aac. ccg gca tct tgg tct. atg aaa gtt acc 96Ile. Ile Ala Ala Tyr Gln. Asn Pro Ala Ser Trp. Ser Met Lys Val Thr. 20 25 30 gtt gct ttc. aac cag ttc ggt ccg. ttc gca tct aaa aac. ttc cat ctg 144 Val. Ala Phe Asn Gln Phe. Gly Pro Phe Ala Ser. Lys Asn Phe His Leu. 35 40 45 cag aaa aac. acc aaa ctg acc tct. ggt aaa atc gca tct. tgc ctg aac 192 Gln. Lys Asn Thr Lys Leu. Thr Ser Gly Lys Ile Ala Ser Cys Leu Asn 50 55 60 tac ggt ctg gtt cat gtt gca aac aac aac tac gac ccg tct ggt aaa. 240 Tyr Gly Leu Val. His Val Ala Asn Asn. Asn Tyr Asp Pro Ser. Gly Lys 65 70 75 tac. gaa ggt ggt aac atc. tac acc aaa aaa gaa. gca ttc aac gtt gaa. 288 Tyr Glu Gly Gly. Asn Ile Tyr Thr Lys. Lys Glu Ala Phe Asn. Val Glu 80 85 90 95. cag ttc gct aaa ctg. acc ggt ttc acc ctg. atg ggt cgt gca gat. ccg 336 Gln Phe Ala. Lys Leu Thr Gly Phe. Thr Leu Met Gly Arg. Ala Asp Pro 100 105. 110 cgt ggt atc atc. gca gca tac cag aac ccg gca tct tgg tct atg aaa 384 Arg Gly Ile Ile Ala Ala Tyr Gln Asn Pro Ala Ser Trp Ser Met Lys 11. 5 120 125 gtt acc gtt. gct ttc aac cag ttc. ggt ccg ttc gca tct. aaa aac ttc 432 Val. Thr Val Ala Phe Asn. Gln Phe Gly Pro Phe. Ala Ser Lys Asn Phe. 130 135 140 cat ctg. cag aaa aac acc aaa. ctg acc tct ggt aaa. atc gca tct tgc 480. His Leu Gln Lys Asn. Thr Lys Leu Thr Ser. Gly Lys Ile Ala Ser. Cys 145 150 155 ctg. aac tac ggt ctg gtt. cat gtt gca aac aac. aac tac gac ccg tct. 528 Leu Asn Tyr Gly. Leu Val His Val Ala Asn Asn Asn Tyr Asp Pro Ser 160 165 170 175 ggt aaa tac gaa ggt ggt aac atc tac acc aaa aaa gaa gc. a ttc aac 576 Gly Lys. Tyr Glu Gly Gly Asn. Ile Tyr Thr Lys Lys. Glu Ala Phe Asn 180. 185 190 gtt gaa

cag. ttc gct aaa ctg acc. ggt ttc acc ctg atg ggt cgt gca 624 Val Glu Gln Phe Ala Lys Leu Thr Gly
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which comprises T cell epitopes derived from cedar pollen allergens -- <400> 12Met Thr Met Ile
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Ala Ser. Trp Ser Met Lys Val. Thr 20 25 30 Val Ala. Phe Asn Gln Phe Gly. Pro Phe Ala Ser Lys.
Asn Phe His Leu 35 40. 45 Gln Lys Asn Thr Lys. Leu Thr Ser Gly Lys. Ile Ala Ser Cys Leu. Asn
50 55 60 Tyr Gly. Leu Val His Val Ala. Asn Asn Asn Tyr Asp. Pro Ser Gly Lys 65 70. 75 Tyr Glu
Gly Gly Asn. Ile Tyr Thr Lys Lys Glu Ala Phe Asn Val Glu 80 85 90 95 Gln Phe Ala Lys Leu Thr
Gly Phe Thr Leu Met Gl. y Arg Ala Asp Pro 100. 105 110 Arg Gly Ile. Ile Ala Ala Tyr Gln. Asn Pro
Ala Ser Trp. Ser Met Lys 115 120. 125 Val Thr Val Ala. Phe Asn Gln Phe Gly. Pro Phe Ala Ser
Lys. Asn Phe 130 135 140. His Leu Gln Lys Asn. Thr Lys Leu Thr Ser. Gly Lys Ile Ala Ser. Cys
145 150 155 Leu. Asn Tyr Gly Leu Val. His Val Ala Asn Asn. Asn Tyr Asp Pro Ser 160 165 170
175 Gly Lys Tyr Glu Gly Gly Asn Ile Tyr Thr Lys Lys Glu Ala Phe Asn 180 185 190 Val Glu Gln
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Asn. Lys Leu Thr Ser Gly. Lys Ile Ala Ser 35 40. 45 Cys Leu Asn Tyr Gly. Leu Val His Val Ala.
Asn Asn Asn Tyr Asp. Pro 50 55 60 Ser Gly. Lys Tyr Glu Gly Gly. Asn Ile Tyr Thr Lys Lys Glu
Ala Phe 65 70 75 80 Asn Val Glu Gln Phe Ala Lys Leu Thr Gly Phe Thr Leu Met Gly Arg 85 90
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acc gtt gct ttc aac cag ttc ggt ccg gac atc ttc gca t. ct 96Lys Val Thr Val. Ala Phe Asn Gln Phe.
Gly Pro Asp Ile Phe. Ala Ser 20 25 30 aaa. aac ttc cat ctg cag. aaa aac aaa ctg acc. tct ggt aaa
atc gca. 144 Lys Asn Phe His. Leu Gln Lys Asn Lys. Leu Thr Ser Gly Lys. Ile Ala 35 40 45 tcc.
tgc ctg aac tac ggt. ctg gtt cat gtt gca. aac aac aac tac gac. 192 Ser Cys Leu Asn. Tyr Gly Leu
Val His. Val Ala Asn Asn Asn. Tyr Asp 50 55 60 ccg. tct ggt aaa tac gaa. ggt ggt aac atc tac acc
aaa aaa gaa gca 240 Pro Ser Gly Lys Tyr Glu Gly Gly Asn Ile Tyr Thr Lys Lys Glu Ala 65 70 7. 5
ttc aac gtt gaa cag. ttc gct aaa ctg acc. ggt ttc acc ctg atg ggt 288 Phe Asn Val Glu Gln Phe Ala
Lys Leu Thr Gly Phe Thr Leu Met Gly 80 85 90 95 cgt 291 Arg -- <210> 15<211> 97<212>
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which consists of T cell epitopes. derived from cedar pollen. allergens <400> 15Met. Gly Ile Ile
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Ser Gly Lys Ile Ala 35 40 45 Ser Cys Leu Asn Tyr Gly Leu Val His Val Ala Asn Asn Asn Tyr Asp
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Pro Ser Gly Lys Tyr Glu Gly Gly Asn Ile Tyr Thr Lys Lys Glu Ala 65 70 75 80 ttc aat gtt gaa cgt gca gat ctg taagtcca. c 274 Phe Asn Val Glu Arg Ala Asp Leu 85 -- < 210> 25<211> 88<212> PRT<213> Artificial Sequence <220> <223> Description of Artificial Sequence:Designed peptide which consists of T cell epitopes. derived from cedar pollen. allergens <400> 25Asp. Pro Arg Gly Ile Ile. Ala Ala Tyr Gln Asn. Pro Ala Ser Trp Ser. 1 5 10 15 Met Lys Val. Thr Val Ala Phe Asn. Gln Phe Gly Pro Phe. Ala Ser Lys 20 25 30. Asn Phe His Leu Gln. Lys Asn Thr Lys Leu. Thr Ser Gly Lys Ile Ala 35 40 45 Ser Cys Leu Asn Tyr Gly Leu Val His Val Ala Asn Asn Asn Tyr Asp 50 55 60 Pro Ser Gl. y Lys Tyr Glu Gly Gly Asn Ile Tyr Thr Lys Lys Glu Ala 65 70 75 80 Phe Asn Val Glu Arg Ala Asp Leu 85 <210> 26<211> 14<212> PRT<213> Artificial Sequence <220> <223>Description of Artificial Sequence: Linker peptide <400> 26Thr Met Ile Thr Asn Ser Ser Ser Val Pro Gly Asp Pro Arg 1 5 10 -- < 210> 27<211> 5<212> PRT<213> Artificial Sequence <220> <223> Description of Artificial Sequence:Linker peptide <400> 27 Arg Ala Asp Pro Arg 1 5 <210> 28 <211> 310<212> DNA -- < 213> Artificial Sequence <220> <223> Description of Artificial Sequence: Designed DNA encoding a polypeptide which consists of T cell epitopes derived from cedar pollen allergens -- < 220> <221> CDS<222> (11)..(289) <400> 28ggatccgcgt ggt atc atc gca gca tac cag aac ccg gca tct tgg tct 49 Gly Ile Ile Ala Ala Tyr Gln Asn Pro. 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Ser Lys 15 20 25 aac. ttc cat ctg cag aaa. aac acc aaa ctg acc. tct ggt aaa atc gca. 145 Asn Phe His Leu. Gln Lys Asn Thr Lys Leu Thr Ser Gly Lys Ile Ala 30 35 40 45 tct tgc ctg aac tac ggt ctg gtt cat gtt gca aac aa. c aac tac gac 193 Ser. Cys Leu Asn Tyr Gly. Leu Val His Val Ala. Asn Asn Asn Tyr Asp. 50 55 60 ccg tct ggt. aaa tac gaa ggt ggt. aac atc tac acc aaa. aaa gaa gca 241 Pro. Ser Gly Lys Tyr Glu. Gly Gly Asn Ile Tyr Thr Lys Lys Glu Ala 65 70 75 ttc aac gtt gaa cgtgcagatc tgtaagtcca c 274 Phe Asn Val Glu 80 -- < 210> 31<211> 81<212> PRT<213> Artificial Sequence <220> <223> Description of Artificial Sequence:Designed peptide which consists of T cell epitopes. derived from cedar pollen. allergens <400> 31Gly. Ile Ile Ala Ala Tyr. Gln Asn Pro Ala Ser Trp Ser Met Lys Val 1 5 10 15 Thr Val Ala Phe Asn Gln Phe Gly Pro Phe

Ala Ser Lys Asn Phe His 20 25 30 Leu Gln Lys Asn Thr Lys Leu Thr Ser Gly Lys Ile Ala Ser Cys
 Leu 35 40 45 Asn Tyr Gly Leu Val His Val Ala Asn Asn Asn Tyr Asp Pro Ser Gly 50 55 60 Lys
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 Phe Ala Ser Lys Asn 35. 40 45 ttc cat ctg cag. aaa aac aaa ctg acc. tct ggt aaa atc gca. tcc tgc
 192 Phe His. Leu Gln Lys Asn Lys. Leu Thr Ser Gly Lys. Ile Ala Ser Cys 50 55. 60 ctg aac tac
 ggt ctg. gtt cat gtt gca aac. aac aac tac gac ccg. tct 240 Leu Asn Tyr. Gly Leu Val His Val. Ala
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 aaa ctg acc tct. ggt aaa 480 Ala Ser. Lys Asn Phe His Leu. Gln Lys Asn Lys Leu. Thr Ser Gly
 Lys 145. 150 155 atc gca tcc. tgc ctg aac tac ggt. ctg gtt cat gtt gca. aac aac aac 528 Ile. Ala
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 aaa tac gaa ggt ggt aac atc tac acc aaa aaa. 576 Tyr Asp Pro Ser. Gly Lys Tyr Glu Gly. Gly Asn
 Ile Tyr Thr. Lys Lys 180 185 190. gaa gca ttc aac gtt. gaa cag ttc gct aaa. ctg acc ggt ttc acc.
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 Ala Ser Lys Asn 35 40 45 Phe His Leu Gln Lys Asn Lys Leu Thr Ser Gly Lys Ile Ala Ser Cys 50
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 Thr Gly Phe Thr Leu. Met Gly Arg Ala 100. 105 110 Asp Pro Arg. Gly Ile Ile Ala Ala. Tyr Gln Asn
 Pro Ala. Ser Trp Lys 115 120. 125 Ser Met Lys Val. Thr Val Ala Phe Asn. Gln Phe Gly Pro Asp.
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 gaacgaccga gcgcagcgag tcagtgcgcg aggaagcgga aga 4613 — < 210> 80<211> 4<212> PRT<213>
 Artificial Sequence <220> <223> Description of Artificial Sequence:Linker peptide <400> 80. Arg
 Ala Asp Leu 1[0189]

[Array table free text]

Array number 1.: peptide—sequence Number 4: peptide sequence number 2: which consists of
 two or more cedar pollen allergen origin T cell epitopes — peptide sequence number 3: which
 consists of two or more cedar pollen allergen origin T cell epitopes — it consists of two or more
 cedar pollen allergen origin T cell epitopes. The peptide—sequence number 5 which consists of
 two or more cedar—pollen allergen origin T cell epitopes: Peptide which consists of two or more
 cedar pollen allergen origin T cell epitopes [0190]Array number 6 : two or more cedar pollen
 allergen origin T cell epitopes. included peptide sequence number 9: — DNA sequence number
 10: which encodes peptide containing two or more cedar pollen allergen origin T cell epitopes —
 peptide sequence number 11: containing two or more cedar pollen allergen origin T cell epitopes
 — two or more cedar pollen allergen origin T cell epitopes. The included peptide. DNA sequence
 number 12: to encode — peptide sequence number 13: containing two or more cedar pollen
 allergen origin T cell epitopes — peptide sequence number 14: containing two or more cedar
 pollen allergen origin T cell epitopes — the peptide which consists of two or more cedar pollen
 allergen origin T cell epitopes. The DNA—sequence number 15 to encode: Peptide which consists
 of two or more cedar pollen allergen origin T cell epitopes [0191]Array number 24.: The DNA
 sequence number 25 which encodes the peptide which consists of two or more cedar pollen
 allergen origin T cell epitopes : Peptide sequence number 26:linker peptide sequence number
 27:linker peptide sequence number 28which consists of two or more cedar pollen allergen origin
 T cell epitopes :. DNA sequence number 29: which encodes the peptide which consists of two or
 more cedar pollen allergen origin T cell epitopes — peptide sequence number 30: which consists
 of two or more cedar pollen allergen origin T cell epitopes — DNA which encodes the peptide
 which consists of two or more cedar pollen allergen origin T cell epitopes [0192]Array number
 31.: peptide sequence number 32: which consists of two or more cedar pollen allergen origin T
 cell epitopes — oligonucleotide array number 33: for building DNA which encodes the peptide
 which consists of two or more cedar pollen allergen origin T cell epitopes — as for plurality, a
 cedar pollen allergen origin T cell epitope. Peptide, ** and others. DNA to encode.
 oligonucleotide array number 34: for building — oligonucleotide array number 35: for building DNA
 which encodes the peptide which consists of two or more cedar pollen allergen origin T cell
 epitopes — the peptide which consists of two or more cedar pollen allergen origin T cell
 epitopes. The oligonucleotide for building DNA to encode [0193]array number 36: —
 oligonucleotide array number 37: for building DNA which encodes the peptide which consists of
 two or more cedar pollen allergen origin T cell epitopes — the peptide which consists of two or
 more cedar pollen allergen origin T cell epitopes. DNA to encode. oligonucleotide array number
 38: for building — oligonucleotide array number 39: for building DNA which encodes the peptide
 which consists of two or more cedar pollen allergen origin T cell epitopes — the peptide which
 consists of two or more cedar pollen allergen origin T cell epitopes. The oligonucleotide array
 number 40 for building DNA to encode: The oligonucleotide for building DNA which encodes the

peptide which consists of two or more cedar pollen allergen origin T cell epitopes [0194]array number 41: — oligonucleotide array number 42: for building DNA which encodes the peptide which consists of two or more cedar pollen allergen origin T cell epitopes — the peptide which consists of two or more cedar pollen allergen origin T cell epitopes. DNA to encode. oligonucleotide array number 43: for building — oligonucleotide array number 44: for building DNA which encodes the peptide which consists of two or more cedar pollen allergen origin T cell epitopes — the peptide which consists of two or more cedar pollen allergen origin T cell epitopes. The oligonucleotide array number 45 for building DNA to encode: The oligonucleotide for building DNA which encodes the peptide which consists of two or more cedar pollen allergen origin T cell epitopes [0195]array number 46: — oligonucleotide array number 47: for building DNA which encodes the peptide which consists of two or more cedar pollen allergen origin T cell epitopes — the peptide which consists of two or more cedar pollen allergen origin T cell epitopes. DNA to encode. oligonucleotide array number 48: for building — oligonucleotide array number 49: for building DNA which encodes the peptide which consists of two or more cedar pollen allergen origin T cell epitopes — the peptide which consists of two or more cedar pollen allergen origin T cell epitopes. The oligonucleotide array number 50 for building DNA to encode: The PCR primer for amplifying DNA which encodes the peptide which consists of two or more cedar pollen allergen origin T cell epitopes[0196]Array number 51: The PCR primer array number 52 for amplifying DNA which encodes the peptide which consists of two or more cedar pollen allergen origin T cell epitopes : DNA sequence number 53which encodes the peptide which consists of two or more cedar pollen allergen origin T cell epitopes : peptide sequence number 54: which consists of two or more cedar pollen allergen origin T cell epitopes — oligonucleotide array number 55: for building DNA which encodes the peptide which consists of two or more cedar pollen allergen origin T cell epitopes — as for plurality, a cedar pollen allergen origin T cell epitope. The oligonucleotide for building DNA which encodes peptide, ** and others [0197]array number 56: — oligonucleotide array number 57: for building DNA which encodes the peptide which consists of two or more cedar pollen allergen origin T cell epitopes — the peptide which consists of two or more cedar pollen allergen origin T cell epitopes. DNA to encode. oligonucleotide array number 58: for building — oligonucleotide array number 59: for building DNA which encodes the peptide which consists of two or more cedar pollen allergen origin T cell epitopes — the peptide which consists of two or more cedar pollen allergen origin T cell epitopes. The oligonucleotide array number 60 for building DNA to encode: DNA which encodes the peptide which consists of two or more cedar pollen allergen origin T cell epitopes [0198] Array number 61: To PCR primer array number 64:pUC18 vector for introducing variation into PCR primer array number 63:pUC18 vector for introducing variation into peptide sequence number 62:pUC18 vector which consists of two or more cedar pollen allergen origin T cell epitopes, variation. The PCR primer for introducing variation into PCR primer array number 65:pUC18 vector for introducing [0199]To Array number 66:pUC18M vector, variation. To PCR primer array number 69:pUC18M vector for introducing variation into PCR primer array number 68:pUC18M vector for introducing variation into PCR primer array number 67:pUC18M vector for introducing, variation. The PCR-primer array number 70 for introducing: Plasmid vector pUC18M5 [0200]array number 71: — PCR primer array number 72: for amplifying DNA which encodes the peptide which consists of two or more cedar pollen allergen origin T cell epitopes — DNA which encodes the peptide which consists of two or more cedar pollen allergen origin T cell epitopes. PCR primer array number 73: for amplifying — PCR primer array number 74: for amplifying DNA which encodes the peptide which consists of two or more cedar pollen allergen origin T cell epitopes — the peptide which consists of two or more cedar pollen allergen origin T cell epitopes. The PCR primer for amplifying the fragment in the PCR primer array number 75:pUC18M5 vector for amplifying DNA to encode [0201]The fragment in Array number 76:pUC18M5 vector. PCR primer array number 77for amplifying : The PCR-primer array-number 79:plasmid pUCM5-6h73 array number 80 for amplifying the fragment in the PCR-primer array-number 78:pUC18M5 vector for amplifying the fragment in pUC18M5 vector: Linker peptide

[Translation done.]